Nemertean nervous system
a comparative analysis

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1 Introduction

Nemerteans are predominantly marine animals; most described species are benthic and only a few are pelagic. Some species also invaded a freshwater environment or moist terrestrial habitats (Gibson 1972). Today about 1300 species are described (Kajihara 2008). Most marine nemerteans are highly active benthic hunters (Nordhausen 1995, Thiel 1998, Thiel Kruse 2001) which spend the daytime hidden in the sediment or under stones and hunt their prey at night at low tide (Nordhausen 1995). Nemerteans often follow the tracks of their bait (Amerongen & Chia 1982) and as soon as they detect a prey organism they catch it with a unique structure, their eversible proboscis. The bait is intoxicated by venom glands situated on the proboscis and swallowed as a whole. In some species the cuticle of a prey organism is punctured by a stylet on the proboscis. Since nemerteans are foraging hunters and show an elaborate mating behavior (Bartolomaeus 1984, Thiel & Junoy 2006), they need to have a well developed nervous system and a variety of different sense organs which allow the animals to interact with their environment. The prominent brain and most of the sensory structures are situated in the front part of the animal. Sensory structures comprise the frontal organs, cerebral organs and a number of epidermal sensory cells (Gibson 1972). The sensory cells in the epidermis are supposed to have a tactile function (Gibson 1972), while the frontal organs and the cerebral organs, which may be very well developed in some species, are supposed to have a chemoreceptive function (Scharrer 1941, Ling 1969, 1970, Ferraris 1985). Pigment cup eyes of which up to hundred may be present in one animal (Bürger 1895, Gibson 1972) serve as photoreceptors (von Döhren & Bartolomaeus 2007).

Comparative anatomical studies on nemertean nervous system had been performed in the late 19th century by McIntosh (1873), Hubrecht (1887) and most notably Bürger (1895). Later researchers did not focus on the detailed design of the adult nemertean nervous system in comparative analyses. In a much approximated view nemertean nervous system consists of two pair of cerebral ganglia (brain) and two lateral nerve cords.

The brain consists of two dorsal and ventral lobes, and their corresponding commissural tracts. The commissural tracts lie above or beneath the rhynchocoel- or daeum and thus form a ring around it. The central nervous system (brain and lateral medullary cords) is composed of an inner neuropil with an outer layer of surrounding neuronal cell somata. The composition of the brain is typical for spiralians (Richter et al. 2010). A pair of lateral longitudinal medullary cords arise from the ventral lobes of the brain and run along the full body length to the posterior of the animal where they unite again by an anal commissural tract. The neuronal cell somata may be separated from the neuropil of the nerve cells by a layer of extracellular matrix, the inner neurilemma. The entire central nervous system may be enclosed by an outer neurilemma (Bürger 1895, Turbeville & Ruppert 1985, Turbeville 1991). Further nerves belonging to the peripheral nervous system are the cephalic- esophageal nerves and the proboscidial nerves. In some species such as Cephalothricidae dorsal or ventral nerves are present. These nerves are not covered by neuronal cell somata.

The location of the central nervous system in relation to the body wall musculature is supposed to be a valuable character to infer nemertean ingroup relationship (Gibson 1972). In some palaeonemerteans such as *Carinina ochracea* and *Tubulanus* the brain and lateral nerve cords are situated in or just beneath the epidermis (fig. 1a). In other palaeonemerteans such as *Carinoma* and Cephalothricidae the central nervous system is located in the musculature. In hetero- and hoplonemerteans the central nervous system shows an inwards migration and is located inside the circular muscle layer or in the body wall (fig. 1b).

Relationship among nemerteans is still under debate. Formerly nemerteans fell into two higher taxa, the Anopla (without spear (=stylet)) and Enopla (spear bearing) (Schultze 1853, Coe 1943, Gibson 1972). This classification was based on fact that one or several stylets are present on the proboscis. The anopla fell into two higher taxa, the palaeonemerteans, which are supposed to be the most basal group, and the heteronemerteans. Enopla comprised Hoplonemertea and Bdellonemertea (fig. 2). Today palaeonemerteans are not regarded as monophyletic and new higher taxa have been introduced (Sundberg et al. 2001, Thollesson & Norenburg 2003). The heteronemerteans and the former palaeonemertean taxon *Hubrechtella* form a monophyletic clade called Pilidiophora, due to a certain larva form, the pilidium. They are the sister group to the remaining nemerteans (Enopla). Pilidiophora and Neonemertea are the sister group of all palaeonemerteans (Andrade et al., 2011) or, if the palaeonemerteans are a paraphyletic group (Thollesson & Norenburg 2003), to one of the palaeonemertean taxa. The latest molecular analysis (Andrade et al. 2011) also recovers Neonemertea and Pilidiophora as well as Heteronemertea and Hoplonemertea as monophyletic.

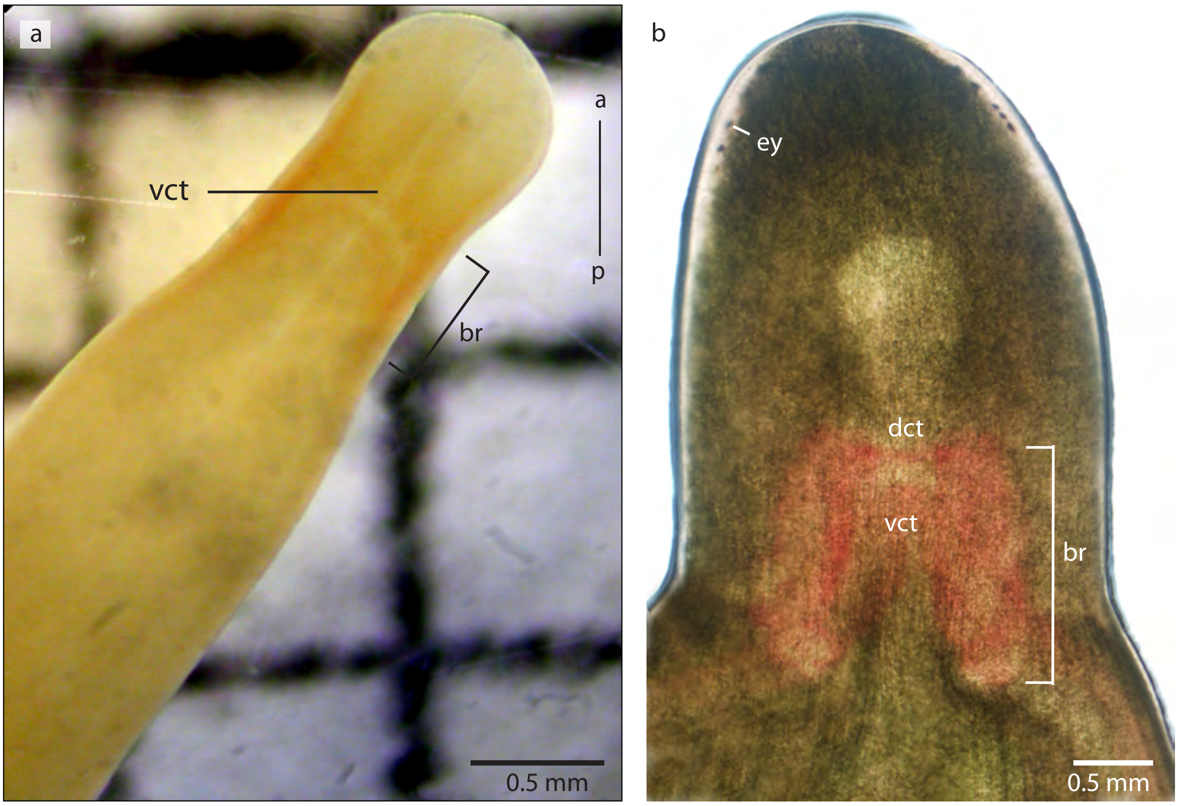


Fig. 1: Living specimen of a: *Carinina ochracea* (Palaeonemertea). The brain (*br*) is located on the outer margins of the head. *a*: anterior, *p*: posterior, *vct*: ventral commissural tract. b: *Lineus viridis* (Heteronemertea) slightly squeezed. The brain (*br*) is located inside the musculature. *ey*: eye, *dct*: dorsal commissural tract.

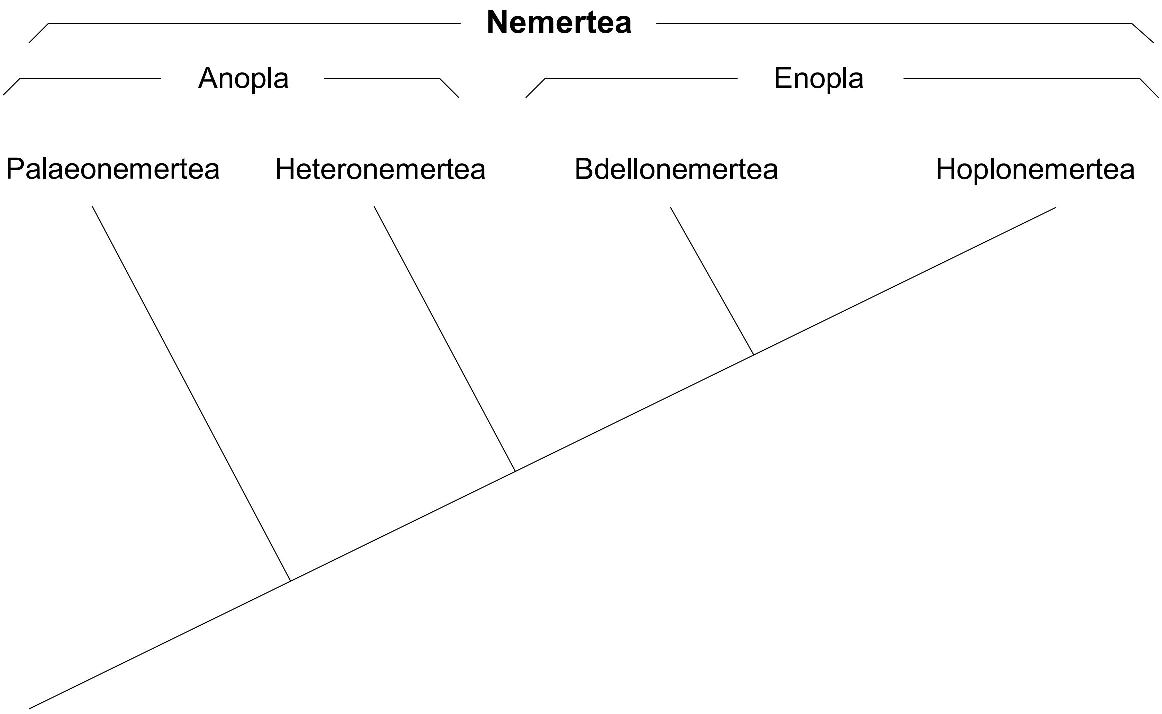


Fig. 2: Phylogenetic tree of nemerteans showing the classification after Coe 1943

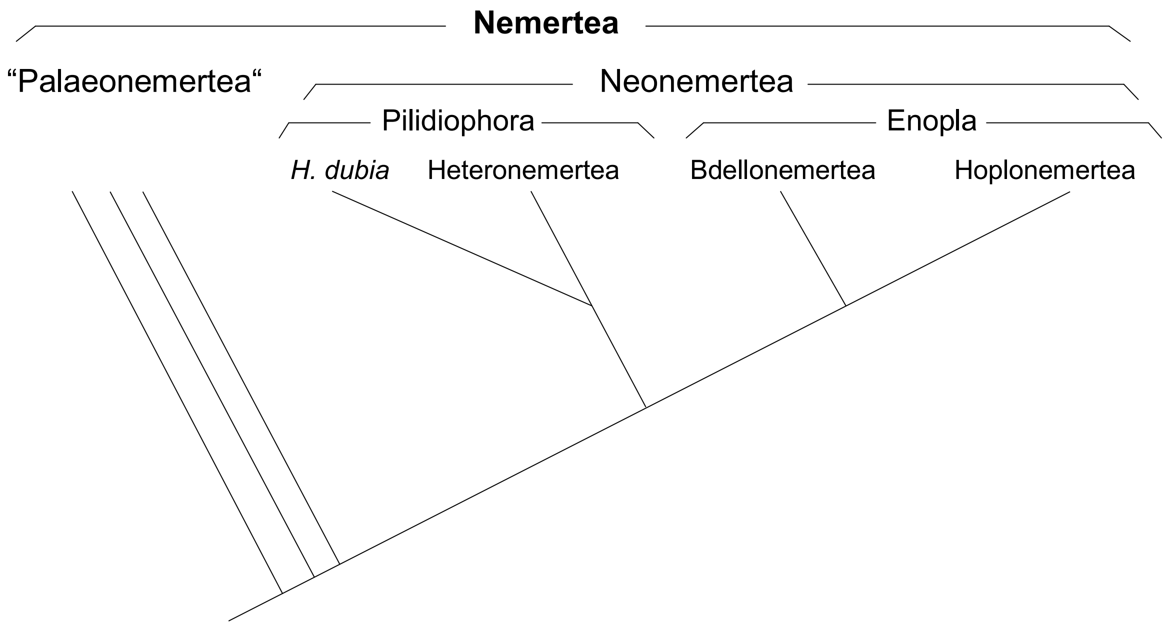


Fig. 3: Phylogenetic tree of nemerteans showing the classification after Tholleson & Norenburg 2003 (simplified).

Nemerteans are spiralians, but their position inside the lophotrochozoan tree is still unclear and under discussion. Traditionally nemerteans were regarded as being closely related to Platyhelminthes because of similarities in the origin of the nervous system and the ciliation of the larvae (e.g. Bürger 1895, Hyman 1951, Nielsen 2001). Recent studies however regard nemerteans as being closely related to brachiopods, annelids or molluscs (Zrzavý et al. 1998, Giribet et al. 2000, Dunn et al. 2008, Heijnol et al. 2009, Paps et al 2009). These studies were primarily based on molecular data, and deeper insight into the relationship among spiralians based on morphology is still wanting.Since a few years now a lot of studies dealing with the nervous system of different spiralian taxa have been available (e.g. Heuer & Loesl 2007, Rothe & Schmidt- Rhaesa 2008, Wollesen et al. 2008, Richter et al. 2010, Heuer et al 2010). The nervous system of an adult nemertean was recently reinvestigated with histological and immunohistochemical methods (Beckers et al. 2011). But the species described there (*Lineus viridis*, Heteronemertea) is supposed to show derived characters.

The aim of this study is to provide a comprehensive comparative analysis of the nervous system of nemerteans. The description of the morphological elements of the nervous system is used to generate a character based data matrix to infer nemertean phylogeny and the relationship of nemerteans to other members of the spiralians.

To be able to infer the position of nemerteans inside the spiralian tree several specimens which are supposed to show the ancestral state of nemertean phylogeny were investigated. This was done to get more information about the morphological elements present in the ground pattern of the nervous system in nemerteans. Additionally specimen which are supposed to show derived characters were investigated to be able to infer ingroup relationship of nemerteans. Because of the comparability of morphological structures it is very important to use standardized methods and markers, all animals were investigated using the same techniques. Classical histological methods and Azan staining were chosen to be able to include in the future data of older literature into the matrix. To get an overall impression of the central nervous system, additionally 3d reconstructions of several specimens were performed. Immunohistochemical methods were applied to reveal the anatomy of minor nerves and the peripheral nervous system. To be able to compare data of recent publications, the antibodies chosen (α-tubulin, serotonin and FMRF-amid) represent the standard markers in most studies.

2 Material & Methods

2.1 Animals

Carinoma mutabilis Griffin, 1898*; Amphiporus imparispinosus* Griffin, 1898 were found in March 2007 in Friday harbor, Cattle Point, San Juan Island, WA, USA. *Procephalothrix filiformis* Johnston, 1828; *Amphiporus lactifloreus* Johnston, 1828, were found on the isle of Sylt in February 2009. *Callinera monensis* Rogers, Gibson & Thorpe, 1992; *Carinina ochracea* Sunberg et al. 2009 were found in Pouldohan/ Brittany France in September 2009. *Riseriellus occultus* Rogers, Junoy, Gibson & Thorpe, 1993; *Emplectonema gracile* Johnston 1837; *Nemertopsis bivittata* Delle & Chiaje 1841 were found near Concarneau/ Brittany France in September 2009. *Cephalothrix linearis* Rathke, 1799; *Tubulanus polymorphus* Renier 1804; *Tubulanus superbus* Kölliker 1845; *Oerstedia dorsalis* Abildgaard, 1806; *Prosorhochmus claperedii* Keferstein, 1862 and *Lineus ruber* Müller, 1774 were found in May 2010 in Roscoff/ Brittany France.

2.2 Histology

The animals were anesthetized in a 7.5% $MgCl_2$- seawater solution in a fridge and fixed overnight in Bouin´s fixative modified after Dubosque- Basil. The animals were completely dehydrated in an ethanol series, followed by incubation in methylbenzoat and butanol. Afterwards the animals were preincubated in Histoplast (Thermo Scientific, Dreieich, Germany) at 60° C for three days with several changes of the medium and finally embedded in Paraplast (McCormick Scientific, Richmond, USA). The different incubation times were changed depending on the size of the animal.

Sections of 5 µm thickness were made using a microtome (Autocut 2050, Reichert-Jung, Leica, Wetzlar). Sections were transferred to glass slides coated with albumen-glycerin.

Slices were stained with Carmalaun subsequently differentiated with sodium phosphotungstate (5%), washed in distilled water and stained aniline blue orange G. Afterwards the slices were embedded with Malinol (Waldeck, Münster, Germany).

Azan stains the neuropil of the nervous system gray. The nuclei of the neuronal cell somata stain red, sometimes with a tinge of orange or purple.. The extracellular matrix (*ecm*, called neurilemma in the central nervous system) stains blue. The musculature stains orange.

2.3 Immunohistochemistry

Animals were anesthetized with a 7.5% $MgCl_2$ solution in seawater in the fridge. The worms were fixed overnight in 4% paraformaldehyde (Electron Microscope Sciences, Hatfield, PA) in seawater (0.1 M). After fixation, the head or middle regions of the animals were washed several times in cold phosphate buffered saline (PBS 0.01 M). The objects were embedded in a gelatine / albumin medium and the blocks were hardened overnight in a 14% formalin solution in the fridge. Animals were cut into sections of 60 µm in thickness with a vibratome (Micron HM 650 V, Thermo Scientific, Dreieich, Germany). The sections were then washed 5 times for 20 minutes in PBS with 0.1% Triton X-100 (Sigma Aldrich, St. Louis, MO, USA) and pre-incubated overnight in a blocking solution of PBS (0,01 M) containing 0.5% TX and 5% normal swine serum (Jackson ImmunoResearch, West Grove, PA). Primary antibodies were added into the blocking solution and incubated overnight at room temperature. The primary antibodies anti- FMRFamide (ImmunoStar, Hudson, WI) and anti-serotonin (Sigma-Aldrich, Saint Louis, MO, USA) were both used at a dilution of 1:10000. Antibodies against acetylated α-tubulin (Sigma Aldrich, St. Louis, MO, USA) were used at a dilution of 1: 500. After incubation with primary antibodies, the sections were again washed 5 times for 20 minutes in PBS with 0.1% TX and were then incubated overnight with the secondary antibody conjugated to fluorophore (Cy3- conjugated goat anti-rabbit; Jackson ImmunoResearch, West Grove, PA) at a dilution of 1:2000 in PBS containing 0.5% TX and 1% normal swine serum. Secondary antibodies against acetylated α-tubulin (Alexa 633, Life Technologies, Darmstadt) were added at a dilution of 1: 250. Subsequently the sections were rinsed again in several changes of PBS containing 0.1% TX and then mounted on chrome alum/ gelatine-coated glass slides under glass cover slips using Elvanol (mounting medium after Rodriguez and Deinhard 1960).

For whole animal immunostaining animals were relaxed in a 7, 5% $MgCL_2$ solution and fixed overnight in 4% paraformaldehyde (Electron Microscope Sciences, Hatfield, PA). Animals were postfixed for 20 minutes in methanol and then washed in several steps of PBS (0,01 M). Afterwards the animals were washed with PBS containing Triton X at a dilution of 0, 1% and preincubated in a blocking solution with NSS (Jackson ImmunoResearch, West Grove, PA). The primary antibodies for anti- FMRFamid and serotonin were added at a dilution of 1: 5000. After the incubation the primary antibodies were washed out with PBS containing Triton X at a dilution of 0, 1%. The secondary antibodies (Cy3 for anti- FMRFamid and anti Serotonin, and Alexa 633 for α-tubulin) were added at a dilution of 1: 2000 and incubated for 24 hours. Afterwards the animals were washed in PBS containing 0.1% Triton X and pure PBS. Then the animals were mounted in Murray clear (2 parts benzyl benzoate, 1 part benzyl alcohol) after having been treated with methanol and isopropanol.

2. 4 Analysis and 3d reconstruction

Azan stained slices were analyzed with an Olympus microscope (BX-51) and photographed with an Olympus camera (Olympus cc12) which was equipped with the dot slide system (2.2 Olympus, Hamburg). Afterwards the slices were aligned with imod (Boulder laboratories, Kremer et al. 1996) and imod align (http://www.q-terra.de/biowelt/3drekon/guides/imod_first_aid.pdf). 3 d reconstructions were performed with Fiji (1.45b) /Trakem and Amira (4.0). The videos were produced using the moviemaker plugin in the Amira (4.0) software.

Immunochemical treated objects were analyzed with a Leica confocal laser scanning microscope (TCSSPE excitation wavelength 488 nm, detection range 500-630 nm was used to detect Alexa 488 fluorescence, excitation wavelength 543 nm, detection range 555-700 nm was used to detect Cy3 fluorescence; excitation wavelength 633 nm, detection range 650-800 nm was used to detect Alexa 633 fluorescence) using the LAS AF 1.6.1 software. Stacks were loaded into Fiji (ImageJ 1.45k plugin) and further processed, using the "maximum projection" tool of the image j software (Macophonics 1.44p). The images were finally processed with Fiji and Photoshop (CS4). Figures were arranged using Illustrator (CS4).

2.5 Phylogenetic analysis

The characters that arose of this investigation were used to create a data matrix based on morphological elements of the nervous system. The matrix was edited with nexus editor (0.5.0) (www.taxonomy.zoology.gla.ac.uk/rod/rod.html). Most parsimonious trees were calculated with TNT (Goloboff et al 2008) using the implicit enumeration and implied weighting settings. K scores were changed from 0.5 to 6.1. Since the length and topology of the trees did not change, the K score was set to 3.0. Bootstrap values (BS) were gained using the standard and traditional search settings. The number of replicates was set to 1000 since any change of replicates did not change the values. Trees were finally processed with win clada (1.00.08, Nixon 1999).

Due to an ongoing debate about the basalmost branching taxon of nemerteans and the unknown sistergroup of nemerteans inside the spiralian tree, several taxon are possibly the most basal branching taxon of nemerteans. Therefore an unrooted tree and two trees rooted with possible basalmost branching nemerteans were calculated.

3 Results

General remarks

The nomenclature to describe the nervous system follows the terminology provided in the review by Richter et al. (2010). Since nemerteans are able to expand or contract their bodies enormously and despite a thoroughly relaxation, the degree of contraction is not equal in all animals. Therefore a description based on morphometric data will be ommitted. In order to illustrate the different parts of the nervous system and to facilitate the reading of this work some generalized schematic drawings were prepared (figs. 4, 5).

Brain. The *brain* consists of a *dorsal* and a *ventral* pair of *lobes* and its interconnecting *commissural tracts* of which the *dorsal* one is located above and the *ventral* one below the rhynchocoel (figs. 4a, b). The *dorsal commissural tract* may be located posterior to the *ventral commissural tract* or vice versa. The *somata* are located peripherally and externally to the central *neuropil*; *somata* and *neuropil* may be separated by an *inner neurilemma* of extracellular matrix (*ecm*)(fig. 4b). The *brain* gives rise to different nerves, a pair of ventro-laterally located *medullary cords* and occasionally to *lobe*-like extensions as well as additional *nerves* and *nerve cords (*fig. 4a*)*.

Central nervous system. The central nervous system is characterized by a clear separation into *neuropil* and *somata*. It consists of the *brain*, the *medullary cords*, the roots of peripheral nerves and certain *lobe*-like extensions as well as *nerve cords* of the brain (fig. 4a, b). The latter two extensions of the *brain* are characterized by the attending *somata* which are not separated from those of the *brain*. *Lobe*-like extension of the brain often go along with the *cerebral organs* of heteronemerteans, *frontal nerve cords* are found in cephalothricid species. Each *ventral brain* lobe tapers posteriorly to a *medullary cord*. The *somata* of the *medullary cord* either form a *cap* above and below the neuropil or an inferior median *cap* (fig. 5b).

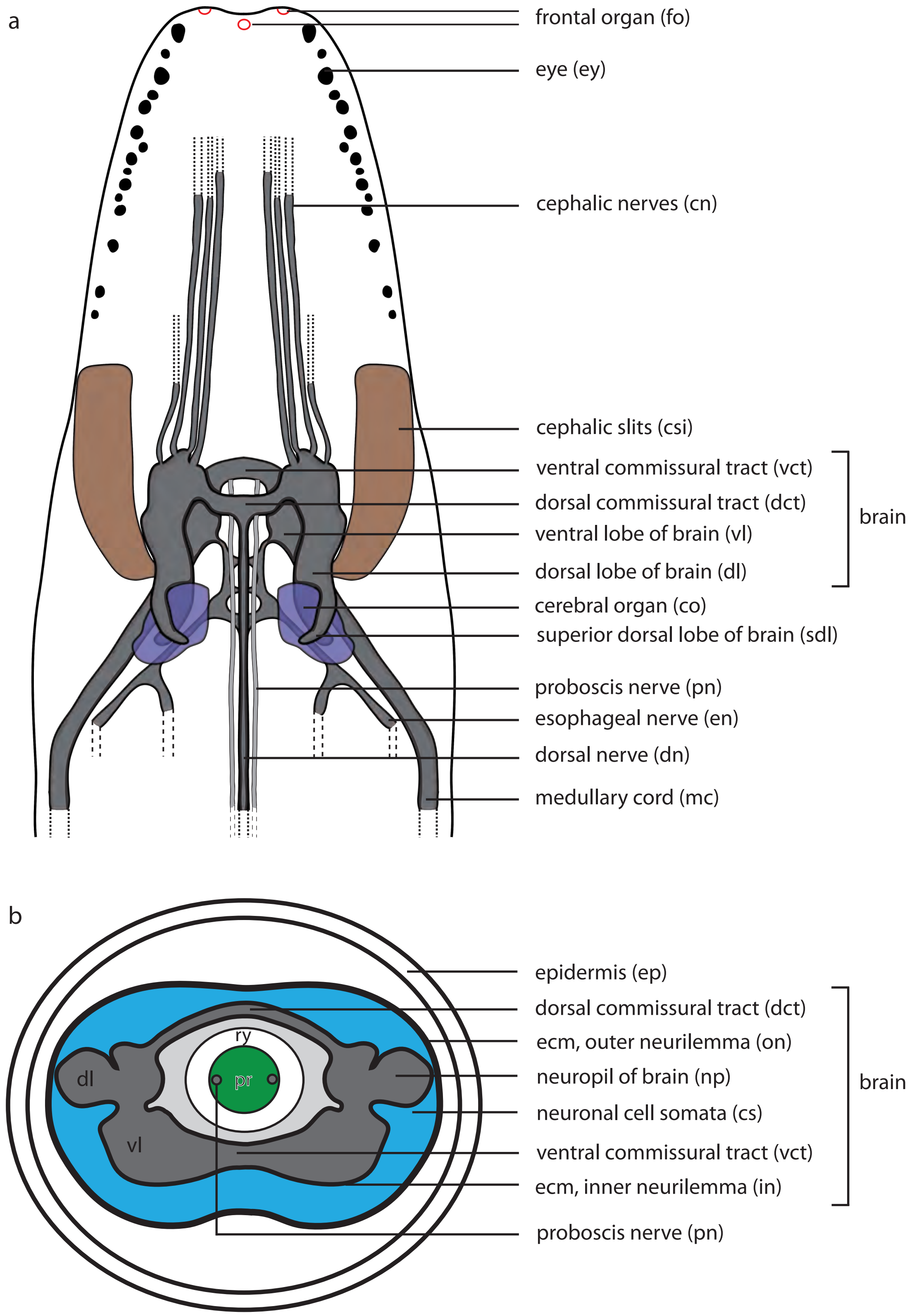


Fig. 4: Schematic drawings of the nemertean central nervous system and its different parts. **a**: Dorsal view of the central nervous system of *Lineus viridis* (Heteronemertea), the neuronal cell somata are not shown (redrawn after Beckers et al. 2011). **b**: Cross section of the brain. The brain is composed of a central neuropil (*gray*) which is surrounded by neuronal cell somata (*blue*). The neuronal cell somata may be separated by an inner neurilemma from the neuropil. The whole brain may be enclosed by an outer neurilemma. The two halves of the brain are interconnected by a dorsal and ventral commissural tract which form a ring around the rhynchocoel (*ry*). *pr*: proboscis

Peripheral nervous system and sensory structures. The peripheral nervous system of nemerteans consists of several minor nerves, such as the *cephalic nerves*, the *proboscis nerves*, *dorsal* and *ventral nerves* and *nerve plexus* (fig. 5). *Nerves* are characterized by bundles of neurites surrounded by an *ecm*, while *nerve plexus* are meshworks of neurites.

- **Nerves**. The medio-caudal section of each *ventral lobe* may give rise to a *ventral nerve* that, depending on the position of the mouth, initially runs ventrally to the rhynchocoel. On the level of the mouth the *ventral nerve* branches into two *esophageal nerves*. These encircle the foregut and merge behind the mouth to continue as *ventral nerve*. A pair of *proboscis nerves* may originate from the *ventral commissural tract* and run posteriorly parallel to the proboscis (fig. 4a). Dorsal and ventral *lobes* of the *brain* give rise to the anterior *cephalic nerves*. A median *dorsal nerve* may originate in the *dorsal commissural tract* and run posteriorly. A *secondary dorsal nerve* may be located ventrally to the *dorsal nerve*. While *ventral* and *dorsal nerves* are *single nerves* that run in a median plane, the *proboscis nerves* and *pharyngeal nerves* may be paired. The latter are situated ventro-laterally of the foregut, the *proboscis nerves* are inside the proboscis wall and may be multiplied (fig. 5b).

- **Plexus**. Both *medullary cords* may be interconnected by *commissural plexus* (fig. 5a). Further plexus are inside the proboscis wall (*proboscidial plexus*), in the wall of the rhynchocoel (*rhynchocoelan plexus*), underneath the gut epithelium (*intrastomatogastric plexus*), surrounding the gut musculature (*suprastomatogastric plexus*), underneath the basal lamina (*subepidermal plexus*) and inside the epidermis (*intraepidermal plexus*) (fig. 5a).

- **Sensory structures**. Nemerteans possess several anterior sensory structures, such as the *frontal organ*, *eyes*, and *cerebral organs* (figs. 4a; 6d, e). The *eyes* are visible by their black shading pigment; *cerebral* and *frontal organs* are defined by their morphology and position.

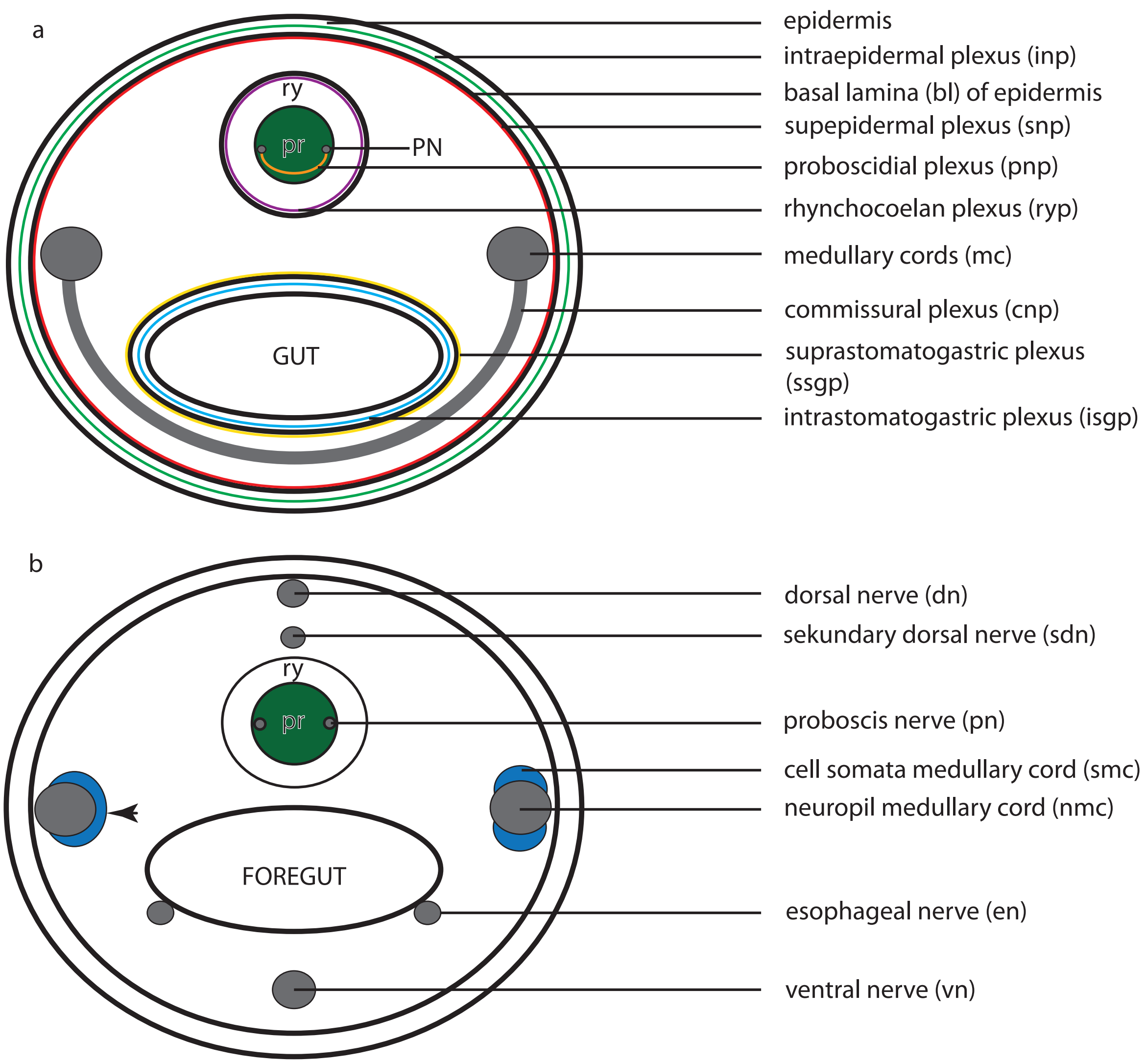


Fig. 5: Schematic drawings of the peripheral nervous system in nemerteans. **a**: Illustration of the different nerve plexus in nemertea. **b**: Illustration of the different major nerves present in nemertea. Note the different location of the neuronal cell somata (*blue*) of the medullary cords. On the left the cell somata are arranged in a u- shaped manner around the neuropil (*arrowhead*). This situation will be referd in the text as cap to the interior. If the somata cover the neuropil the other way round it will be refered as cap to the exterior. On the right the neuronal cell somata overlay the neuropil of the medullary cords dorsally and ventrally. *pr*: proboscis, *ry*: rhynchocoel

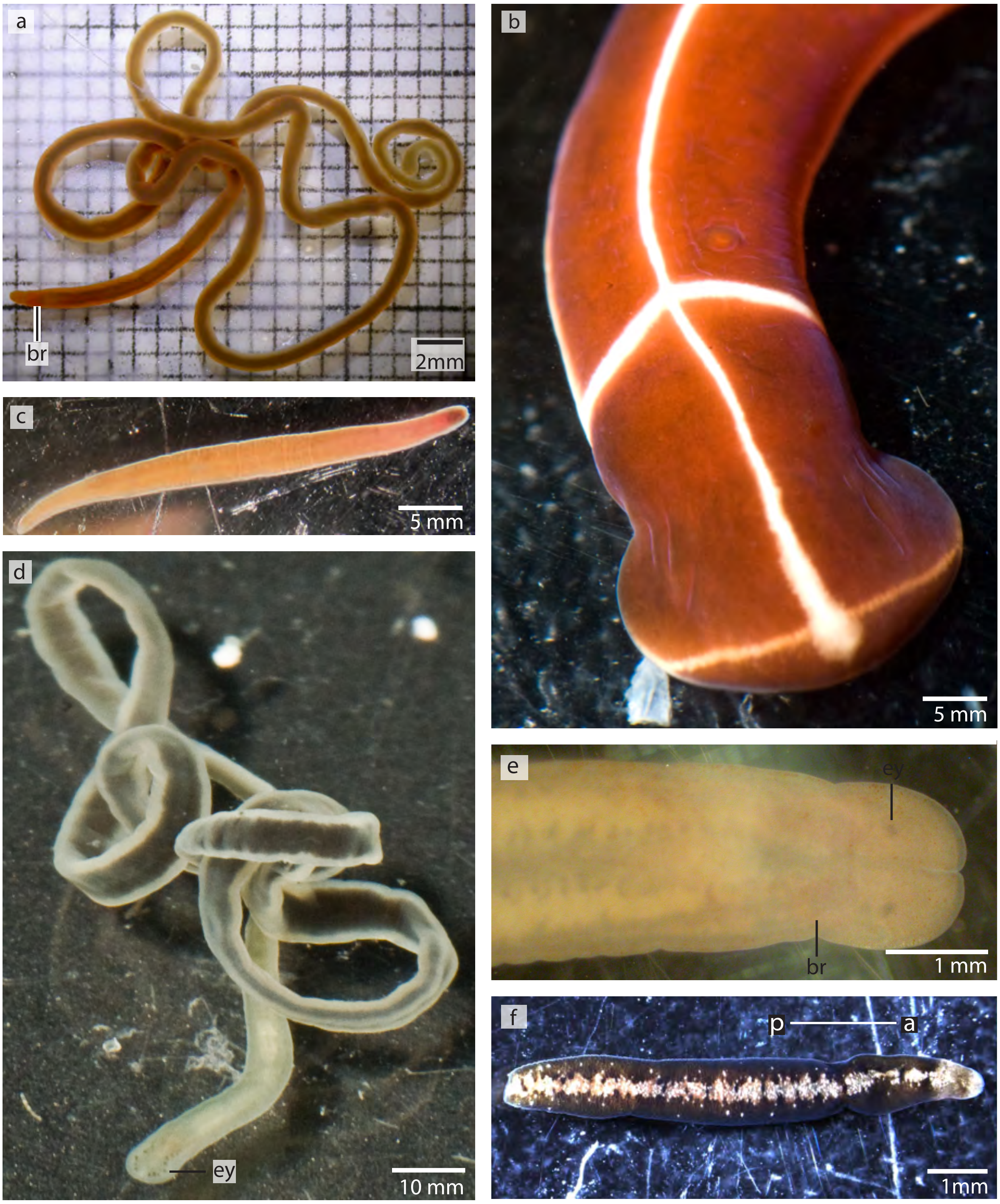


Fig. 6: Living specimen of a: *Cephalothrix linearis br*: brain. b: *Tubulanus superbus* c: *Lineus ruber* d: *Emplectonema gracile ey*: eye e: *Prosorhochmus claperedii br*: brain, *ey*: eye. f: *Oerstedia dorsalis a*: anterior, *p*: posterior.

3.1 Morphological part

3.1.1 *Procephalothrix filiformis* (Cephalothricidae, "Palaeonemertea")

Procephalothrix species are characterized by a long and bluntly pointed head (Gibson 1972) which is termed rostrum. The brain of *Procephalothrix filiformis* is located in the rostrum of the animals and far anterior to the mouth opening (figs. 7a, b). It is embedded in the head musculature.

Brain and central nervous system

Frontally to the brain a massive layer of neuronal cell somata is embedded in the head musculature (figs. 7a; 8a). The somata cover four strands of neuropil which are separated from the somata of the nerve cells by a layer of extracellular matrix (inner neurilemma) (fig. 8b). The nerves lie between the somata and a layer of longitudinal muscles, close to the head lacuna (fig. 8b), and are termed cephalic nerves in literature (Gibson 1972). According to the definitions above (p. 10), these four strands are part of the central nervous system and must be termed nerve cords. These cephalic nerve cords arise from the anterior inner lateral parts of the brain. The dorsal cords are anteriorly bifurcated. The neuronal cell somata of the cephalic cords show no immunoreactivity against FMRF-amid or serotonin (figs. 8a; 10a). In its very posterior part the brain is divided into a dorsal and ventral lobe (fig. 8d). Each lobe is interconnected above or below the rhynchocoel respectively. The ventral commissural tract is more prominent and lies anterior to the dorsal one (figs 7b; 8c). Each dorsal lobe has a bean-shaped structure and ends blindly in a layer of neuronal cell somata. The ventral lobe is confluent with the medullary cords (figs. 7b).

The lateral medullary cords are embedded between layers of longitudinal muscles. They run through the animal and merge again at the tip of the tail of the animal (figs. 7b; 10d). The lateral medullary cords are capped dorsally and ventrally by neuronal cell somata (fig 7f). The neuronal cell somata are separated from the neuropil by *ecm*. However some neurites of the neuropil branch off and run between the cell somata. The neuronal cell somata resemble the neuronal cell somata type 2 in the brain (fig. 7f).

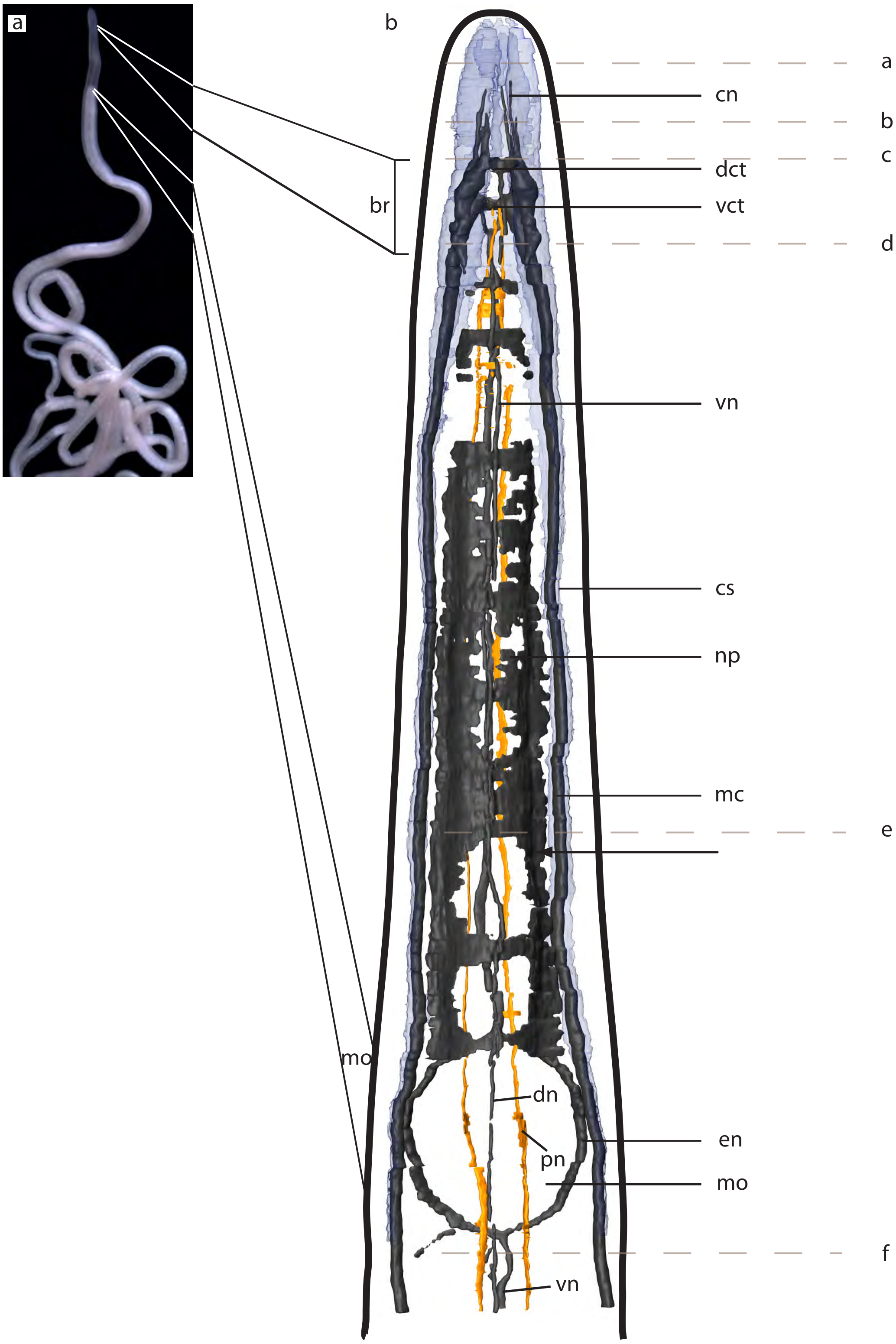


Fig. 7: *Procephalothrix filiformis.* **a**: Living specimen. **b**: Central nervous system, dorsal view, snapshot 3d-reconstruction (501 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are circular arranged around the head of the animals. The paired proboscidial nerves (*pn*, *yellow*) originate from the ventral commissural tract (*vct*). A dorsal nerve (*dn*) originates from the dorsal commissural tract (*dct*), a ventral nerve (*vn*) from the ventral commissural tract. The branching esophageal nerves (*en*) originate from the ventral nerve and surround the mouth opening (*mo*). The lateral medullary cords (*mc*) originate in the ventral lobes of the brain (*br*). Note the neuropil (*np*, *arrow*) which forms a roof-like structure around the rhynchocoel. Letters on the right (**a** - **f**) refer to the histological sections in figure 8.

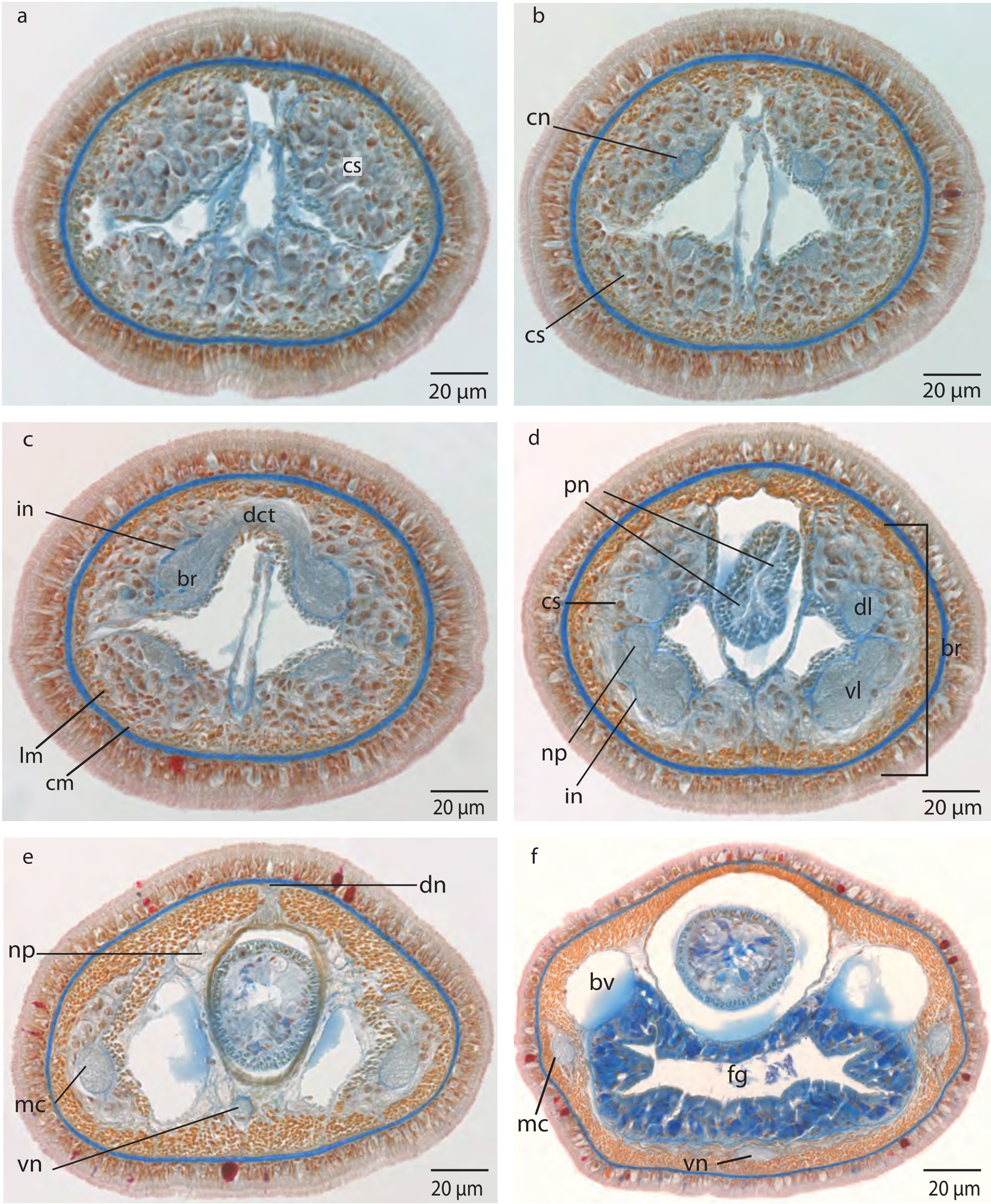


Fig. 8: *Procephalothrix filiformis*, LM, cross sections, Azan. **a**: Frontal part showing the enormous layer of neuronal cell somata (*cs*). **b**: Four cephalic nerves (*cn*) extend towards the tip of the head. **c**: A dorsal commissural tract (*dct*) connects the two halfes of the brain (*br*). The neuronal cell somata are separated from the neuropil of the brain by an inner neurilemma (*in*). The brain is embedded in a layer of longitudinal muscles (*lm*) which lie underneath a layer of circular muscles (*cm*). **d**: The brain (*br*) is divided into a dorsal (*dl*) and ventral (*vl*) lobe in its posterior part; it is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*); the somata are separated from the neuropil by an inner neurilemma (*in*), two proboscidial nerves (*pn*) are present. **e**: A dorsal nerve (*dn*) arises from the dorsal commissural tract, a ventral nerve (*vn*) from the ventral commissural tract, neurites (*ne*) of the dorsal nerve extends in a roof like structure around the rynchocoel. *mc*: medullary cord. **f**: The lateral medullary cords (*mc*) are embedded into the longitudinal muscle layer. *bv*: blood vessel, *fg*: foregut, *vn*: ventral nerve.

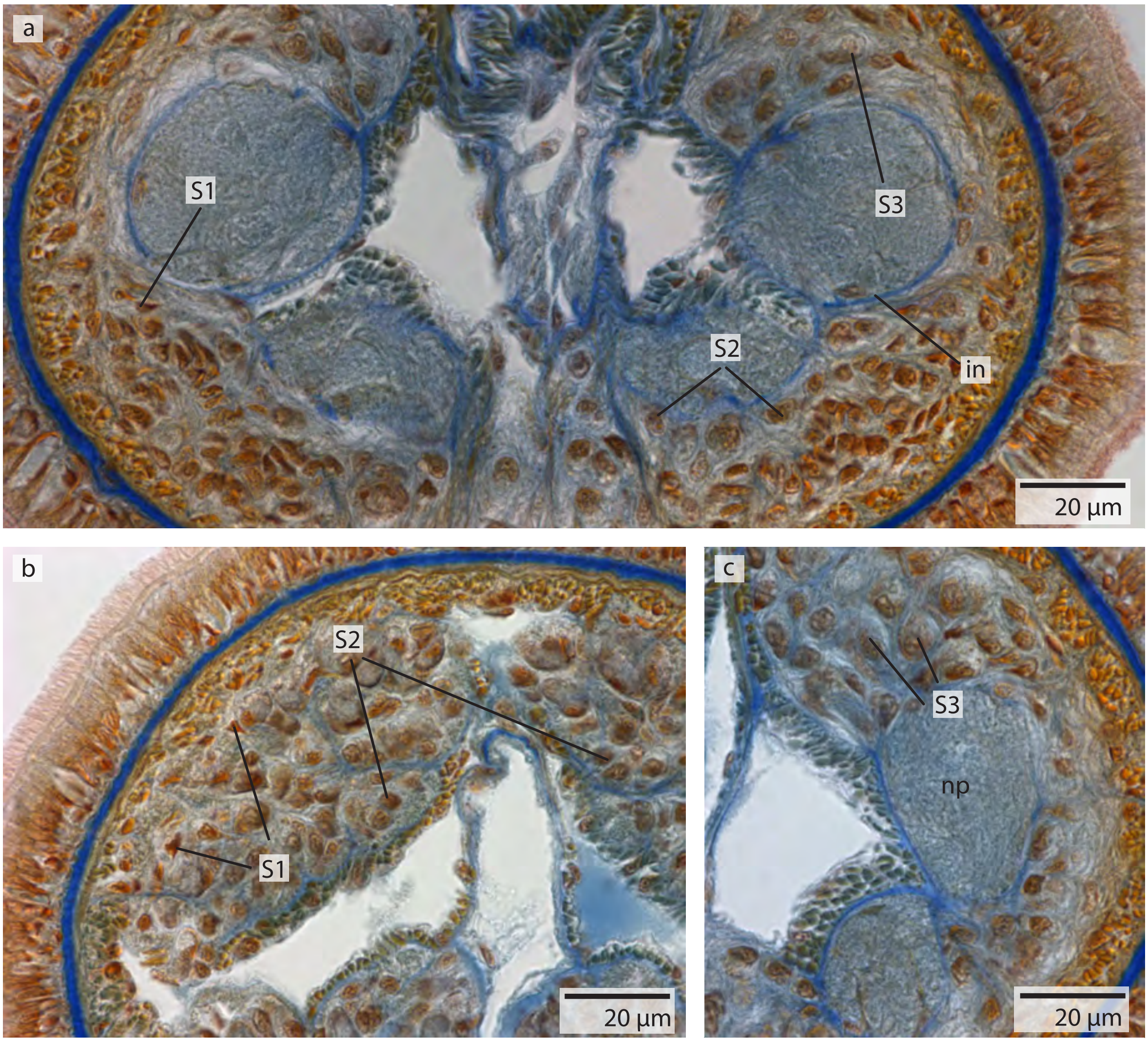


Fig. 9: *Procephalothrix filiformis*, LM, cross sections, Azan. **a**: overview of the brain region showing the location of the different cell somata types 1- 3 (*S1- S3*). *in*: inner neurilemma, *np*: neuropil. **b**: higher magnifcation of *S1*, *S2* showing the cell somata type 1-2 (*S1, S2*). Nuclei of *s1* dye *orange* and the cell bodies are beak shaped. Cell bodies of *S2* are circular and enlarged. Nuclei dye *orange*. **c**: the nuclei of cell somata type 3 (*S3*) also dye *orange*. The cell bodies are circular but more prominent than in *S1-S2*.

In the posterior part of the medullary cords the neuronal cell somata layer decreases. The medullary cords are interconnected by serially arranged small circular neurites that show immunoreactivity against serotonin (fig. 10e).

The brain of *P. filiformis* is composed of a central neuropil which is surrounded by a enormous layer of neuronal cell somata (figs. 7b; 8a- d; 9a- c). There are at least three types of neuronal cell somata discernable (figs. 9a- c). Cell bodies of type 1 neuronal cell somata are slender and beaked. The cells appear all over the brain. Type 2 cells are circular and the cell body is enlarged (fig. 9b). Type 3 cell types are also circular, but the cell body is more prominent than in type 2 cells (fig. 9c). The latter two neuronal cell somata types are evenly distributed along the brain.

Although Azan staining reveals a enormous layer of cell somata in the brain, staining against FMRF marks only very few cells (figs. 10a, b). The neuropil is not only restricted to the central part of the brain (clearly separated by *ecm* from the somata), but is also branching into the neuronal cell somata layer (fig. 8d). The central part of the brain neuropil is separated from the neuronal cell somata bodies by an inner neurilemma (figs. 8d; 9a). An outer neurilemma is not clearly discernable. The neurilemma consists entirely of extracellular matrix (*ecm*) indicated by the blue coloration in the Azan staining.

Minor nerves and peripheral nervous system

In the tip of head four minor nerves are present which show immunoreactivity against serotonin and FMRF-amid. These nerves extend from the tip of the head to the posterior of the animal (figs. 10c; 11a). A circular commissural nerve connects the nerves (fig. 10c).

A dorsal nerve originates from the posterior part of the dorsal commissural tract and runs to the posterior of the animal inner to the outer longitudinal muscle layer. More posterior the dorsal nerve runs beyond the basal lamina of the epidermis. This nerve merges with the lateral medullary cords at the tip of the animals tail (figs. 7b; 8e; 9d).

A ventral nerve is formed by the merger of two nerves that branch off the ventral commissural tract. This ventral nerve runs ventrally to the rhynchocoel until it branches in to form the esophageal nerves. These fuse into the ventral nerve behind the mouth (figs. 7b; 8f; 10a). Staining against FMRF reveals two additional minor nerves which originate behind the mouth (fig. 10a) and run to the posterior of the animal.

There are two main proboscidial nerves which arise from the ventral commissural tract (fig. 7b). The nerves run opposed to each other along both sides of the proboscis and are occasionally joined by a proboscidial plexus. The longitudinal proboscis nerves show immunoreactivity against FMRF, but not against serotonin (figs. 8d; 10c; 10e).

A bright gray staining tissue (nervous plexus) arises from the dorsal nerve and forms a roof-like structure around the rhynchocoel (fig. 7e). The neurites of the intraepidermal plexus show immunoreactivity against FMRF and are arranged in a regular, ladder-like way (fig. 11b). The arrangement of the neurites of other plexus could not be reconstructed.

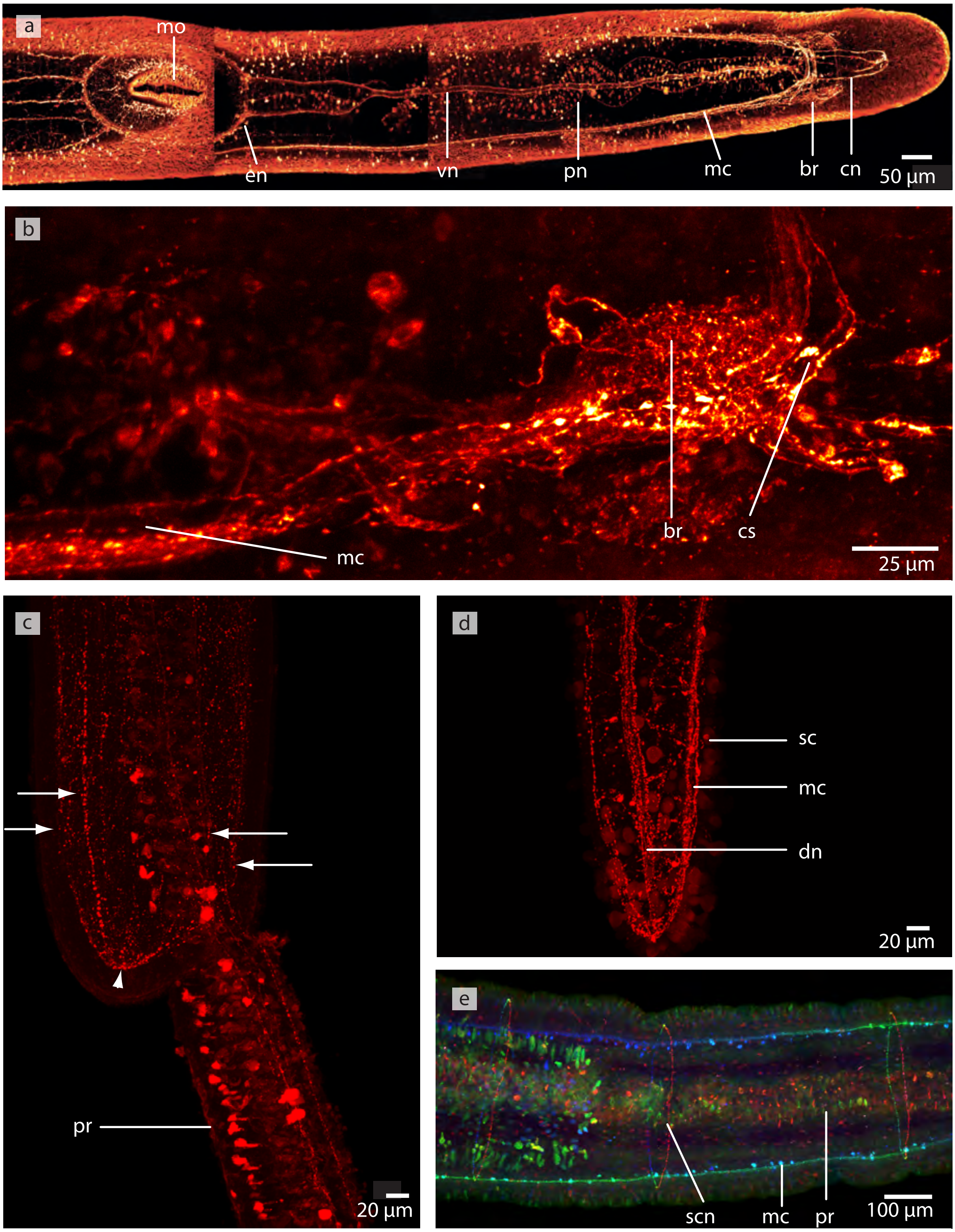


Fig. 10: *Procephalothrix filiformis*, CLSM, whole mount. **a**: Anti FMRF. The cephalic nerves (*cn*) originate in the lateral aspects of the brain (*br*). The lateral medullary cords (*mc*) extend the full body lenghts of the animal. There are two proboscidial nerves (*pn*) which run opposed to each other along both sides of the proboscis. The esophageal nerves (*en*) originate in the ventral nerve (*vn*) and branch short in front of the mouth opening (*mo*). **b**: Anti FMRF. Only few neuronal cell somata (*cs*) of the brain (*br*) are immunoreactive against FMRF. **c**: Anti FMRF. Four minor nerves (*arrow*) unite in the tip of the animals´ head (*arrowhead*). **d**: anti FMRF. The dorsal nerve (*dn*) is connected to the medullary cords (*mc*) in the very posterior part of the animal, bottle shaped sensory cells (*sc*) are distributed all over the body. **e**: Anti serotonin. The medullary cords (*mc*) are connected by serial arranged circular nerves (*scn*). Note that the proboscis nerves show no immunoreactivity against serotonin. *pr*: proboscis.

Sensory structures

Although adult *P. filiformis* react strongly photonegatively (own, unpublished observations) there are no prominent sensory structures present. In the very tip of the head a cluster of cell showing immunoreactivity against FMRF is present (fig. 11c). There are many cells distributed inside the epidermis and all over the body which are immunoreactive against serotonin and FMRF-amid. The cells are bottle-shaped and may represent sensory cells (fig. 10d).

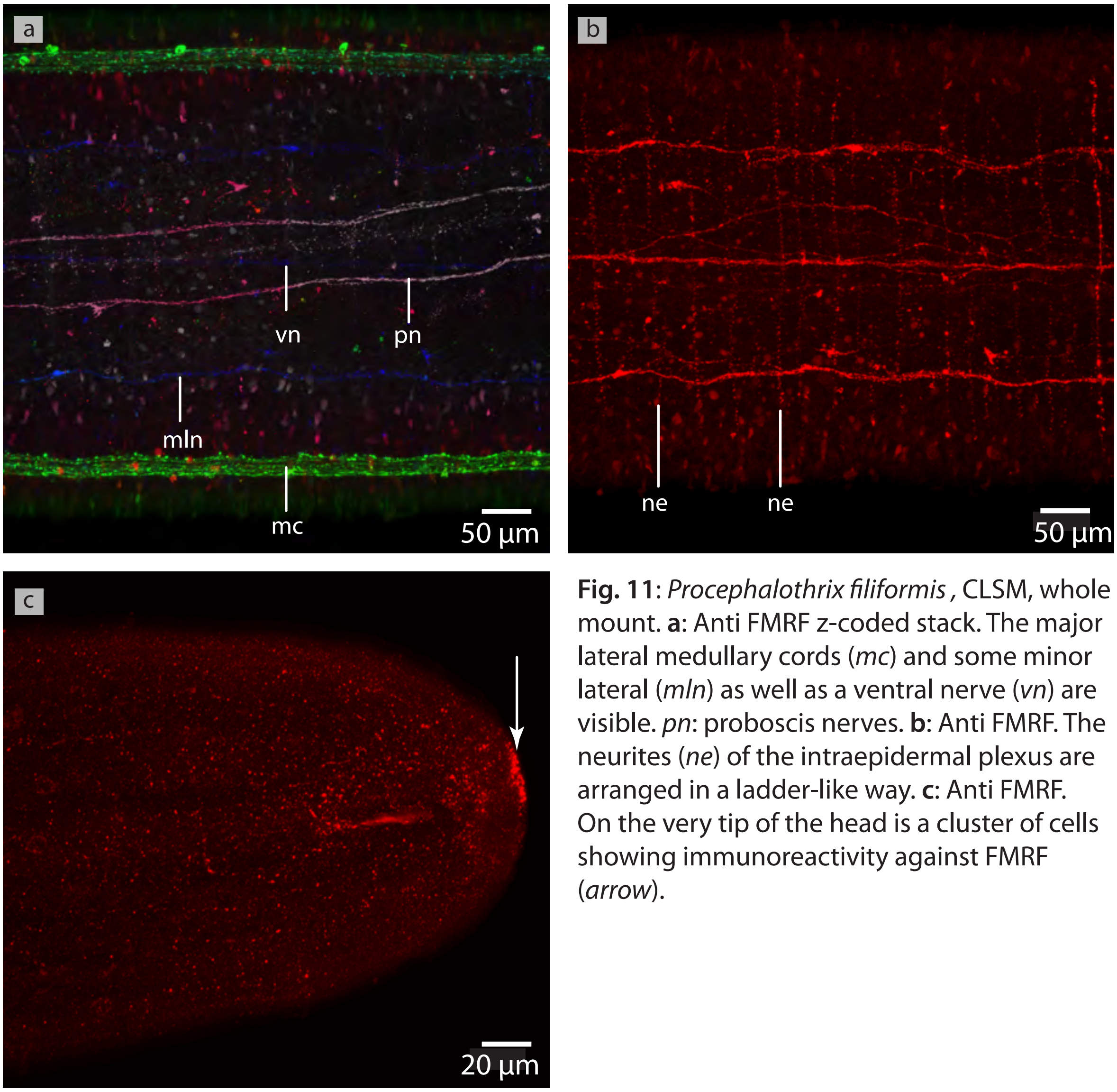


Fig. 11: *Procephalothrix filiformis*, CLSM, whole mount. **a**: Anti FMRF z-coded stack. The major lateral medullary cords (*mc*) and some minor lateral (*mln*) as well as a ventral nerve (*vn*) are visible. *pn*: proboscis nerves. **b**: Anti FMRF. The neurites (*ne*) of the intraepidermal plexus are arranged in a ladder-like way. **c**: Anti FMRF. On the very tip of the head is a cluster of cells showing immunoreactivity against FMRF (*arrow*).

3.1.2 *Cephalothrix linearis* (Cephalothricidae, "Palaeonemertea")

The brain of *Cephalothrix linearis* is located in the rostrum of the animals and far anterior to the mouth opening (figs. 6a; 12a). It is embedded in the head musculature.

Brain and central nervous system

Frontally to the brain a massive layer of neuronal cell somata surrounds four nerves (fig. 12a). The neuronal cell somata are separated by an inner neurilemma from the neuropil. These structures originate from the inner margins of the dorsal and ventral lobe of the brain and are termed cephalic nerves in literature (Gibson 1972). According to the definitions above (p. 10), these four nerves and neuronal cell somata are part of the central nervous system and must be termed nerve cords.

The posterior part of the brain is divided into two parts (figs. 12b, c). Both parts are interconnected by commissural tracts above or below the rhynchocoel, respectively (fig. 12b). The ventral commissural tract is located anterior to the dorsal one and is more prominent. The ventral lobe of the brain is confluent with the lateral medullary cords, the dorsal lobe ends blindly in a layer of neuronal cell somata. The lateral medullary cords run to the posterior of the animal, embedded between the outer circular muscle layer and the longitudinal muscle layer. The neuronal cell somata are separated by an *ecm* to the neuropil of the lateral medullary cords and are of the 1 and 2 type described for the brain (fig. 12d).

The brain of *C. linearis* is composed of a central neuropil which is surrounded by a massive layer of neuronal cell somata (figs. 12b; 13a- c). There are three types of neuronal cell somata discernable (figs. 13a- c). The cell bodies of type 1 neuronal cell somata are small and the nuclei stain in red (figs. 13a- c). The greatest accumulation of these cells is found in the cell somata of the cephalic nerve cords, but they are also found solitarily distributed all over the brain (fig. 13a). Cell bodies of type 2 neuronal cell somata have an oval shape and the nucleus stain dark purple (figs. 13b, c). They are found primarily in the posterior ventral part of the brain and appear there in clusters. The nucleus of type 3 neuronal cell somata stains bright purple (fig. 13c). The cell body of these cells appears circular and enlarged compared to the latter two. These cells are found in the middle part of the brain, just behind the ventral commissural tract. The neuronal cell somata are separated by an inner neurilemma from the neuropil (fig. 13a). An outer neurilemma surrounding the whole parts of the brain was not found.

Minor nerves and peripheral nervous system

A dorsal nerve originates from the dorsal commissural tract and runs to the posterior of the animal beneath the basal lamina of the epidermis.

A ventral nerve arises from the ventral commissural tract. This nerve runs to the posterior and branches into two nerves on the level of the mouth opening. Here the nerve gives rise to the esophageal nerves (fig. 12d) which fuse again behind the mouth opening.

The paired proboscidial nerves originate in the ventral commissural tract of the brain. These nerves are not covered by neuronal cell somata.

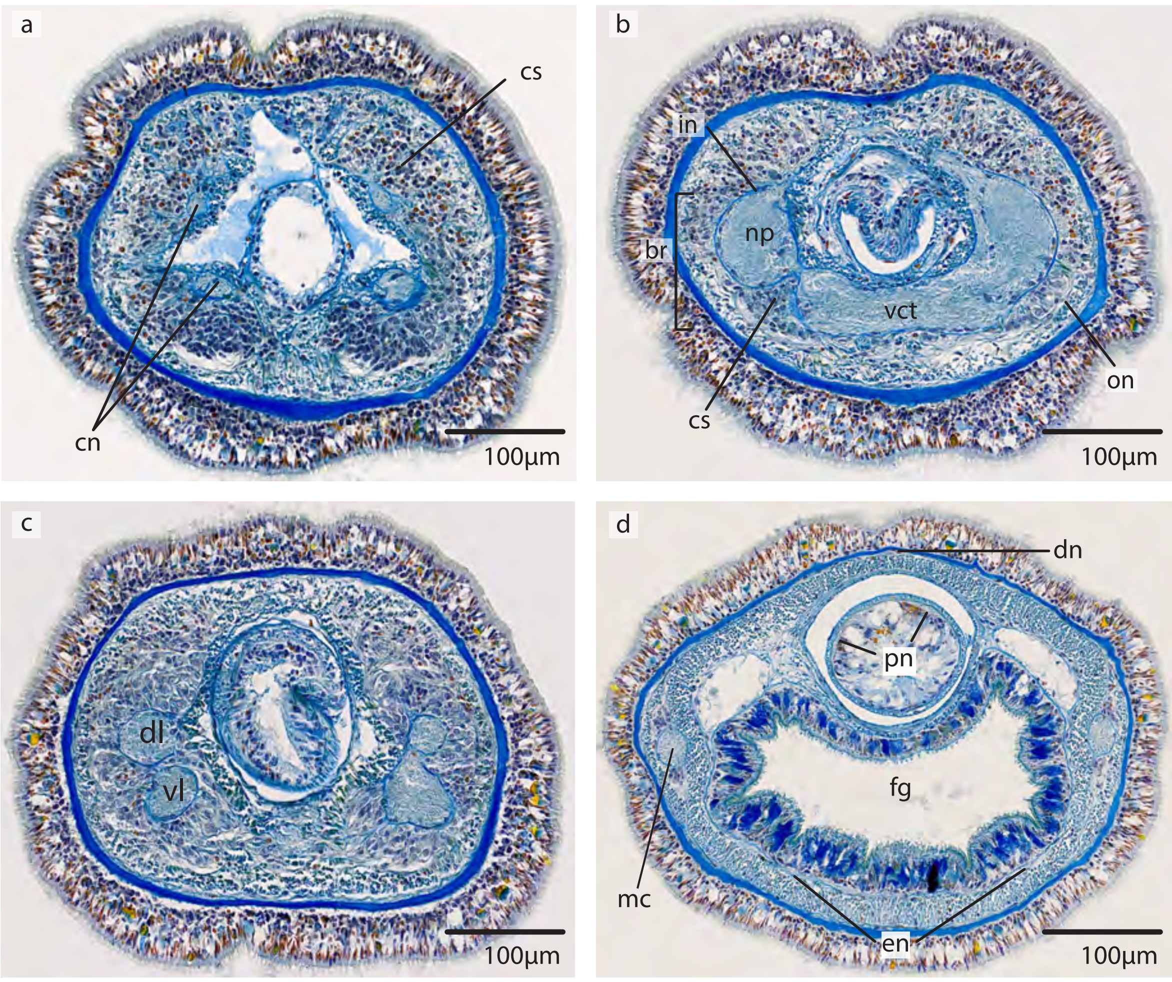


Fig. 12: *Cephalothrix linearis*, LM, cross sections, Azan **a**: The 4 cephalic nerves (*cn*) are frontally covered by a enormous layer of cell somata (*cs*). **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*). The somata are separated from the neuropil by an inner neurilemma (*in*). The whole brain is surrounded by an outer neurilemma (*on*). A ventral commissural tract (*vct*) connects the two parts of the brain. **c**: The posterior part of the brain is divided into a dorsal (*dl*) and ventral (*vl*) lobe. **d**: the ventral lobes are confluent with the lateral medullary cords (*mc*), two proboscidial nerves (*pn*) and esophageal nerves (*en*) are present. A dorsal nerve (*dn*) runs to the posterior underneath the basal lamina of the epidermis. *fg*: foregut.

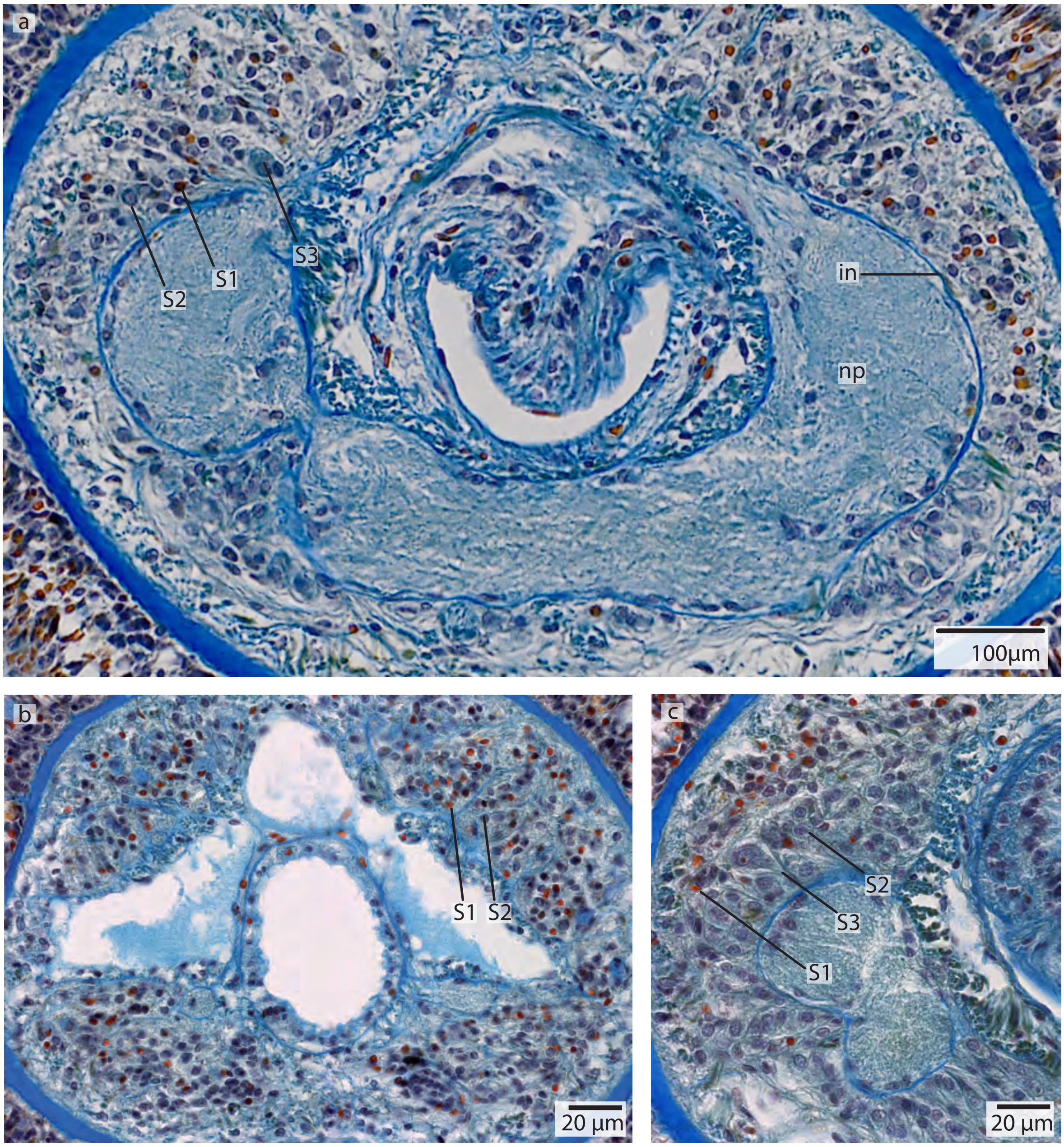


Fig. 13: *Cephalothrix linearis*, LM, cross section, Azan. **a**: Three different neuronal cell somata are discernable (*S1- S3*). The neuropil (*np)* of the brain is separated by an inner neurilemma (*in*) from the neuronal cell somata. **b**: Frontally only cell somata type *1* and type *2* are present. **c**: Nuclei of neuronal cell somata type *1* stain *red*. Nuclei of neuronal cell somata type *2* stain *bright purple* and the cell body is slightly enlarged. The cell body of neuronal cell somata type *3* is the most prominent. The nuclei stain *red*.

Sensory structures

No sensory structures such as eyes or cerebral sense organ are present in *C. linearis*.

3.1.3 *Tubulanus superbus* (Tubulanidae, "Palaeonemertea")

Tubulanid nemerteans are characterized by the sharply demarcated head (Gibson 1972, fig. 6b). The brain of *Tubulanus superbus* is situated close anterior to the mouth opening. It is embedded underneath the basal lamina of the epidermis (subepidermal) and is not externally visible (fig. 6b).

Brain and central nervous system

The very posterior part of the brain is divided into a ventral and dorsal lobe on the level where the duct of the cerebral sense organ gains contact to the environment. The two parts of the brain are connected by commissural tracts (figs. 14b; 14c). The dorsal lobes of the brain are connected by several small dorsal commissural tracts. Whether these tracts are independent of each other or fused is not clearly discernable. The ventral commissural tract is more prominent and is situated posterior to the dorsal ones.

The ventral lobe is confluent with the lateral medullary cords, while the dorsal lobe ends blindly in a layer of neuronal cell somata. The lateral medullary cords run underneath the basal lamina of the epidermis (fig. 14d). The neuronal cell somata of the medullary cords surround the neuropil in a u-shaped manner and cap it to the exterior (fig. 14c). The neuronal cell somata are of type 1 described for the brain. The neuropil of the medullary cords branches into the neuronal cell somata layer.

The brain of *T. superbus* is composed of a central neuropil which is surrounded by neuronal cell somata (figs. 14b; 15a- d). There are at least three types of neuronal cell somata discernable. The nuclei of neuronal cell somata type 1 stain dark purple and cell bodies are small, elongated and they appear in cluster (fig. 15b). The nuclei of type 2 neuronal cell somata stain orange and cell bodies have a drop like appearance (fig. 15bc). These neuronal cell somata are evenly distributed all over the brain. The nuclei of type 3 neuronal cell somata also stain orange and cell bodies have a circular appearance and are more prominent than the latter two (fig. 15bc). These cells are only found in small numbers. The neuronal cell somata are not separated by an inner neurilemma to the neuropil (fig. 15a). A prominent outer neurilemma surrounds the whole brain (fig. 15a). The neurilemma consists entirely of extracellular matrix indicated by the blue coloration in the Azan staining.

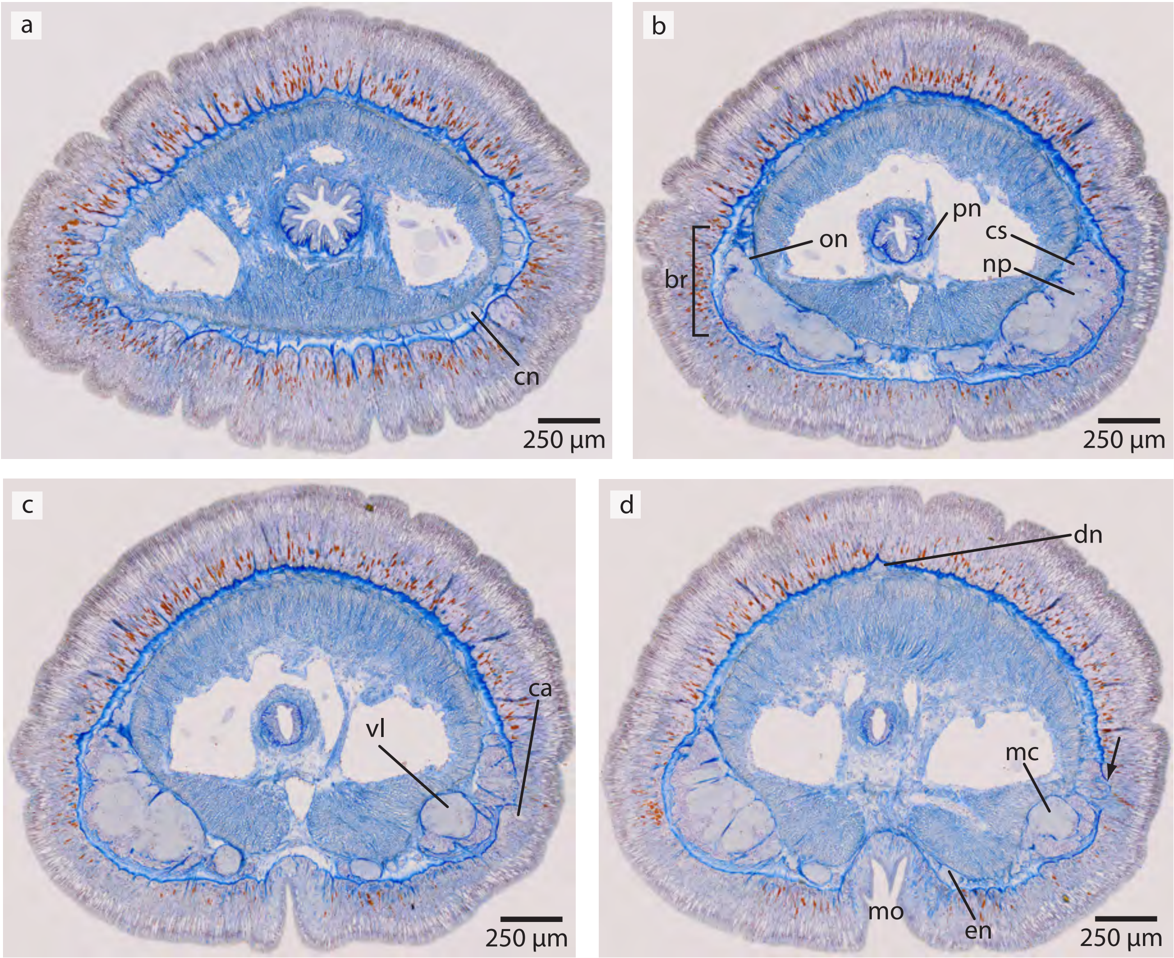


Fig. 14: *Tubulanus superbus*, LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are circular arranged around the margins of the head. **b**: The brain is composed of a central neuropil (*np*) and surrounding cell somtata (*cs*). An outer neurilemma (*on*) surround the whole brain. The two proboscidial nerves (*pn*) originate in the ventral commissural tract. **c**: In its very posterior part the brain is divided into a dorsal and ventral lobe (*vl*). The canal (*ca*) of the cerebral organ does not pierce the basal lamina of the epidermis. **d**: The canal ends in a layer of neuronal cell somata (*arrow*) which have contact to the brain. Two esophageal nerves (*en*) are present. The dorsal nerve (*dn*) originates in a dorsal commissural tract. The ventral lobes of the brain are confluent with the lateral medullary cords (*mc*). *mo*: mouth opening

Minor nerves and peripheral nervous system

The numerous cephalic nerves are embedded between the basal lamina of the epidermis and a layer of longitudinal muscles. The nerves are arranged circularly around the margins of the head (fig. 14a) and separated by a neurilemma from each other. The cephalic nerves are not covered by neuronal cell somata.

A dorsal nerve arises from one of the small dorsal commissural tracts and runs through the animal underneath the basal lamina of the epidermis (fig. 14d). The esophageal nerves (fig. 14d) and the two proboscis nerves arise from the ventral commissural tract. The latter run opposed to each other along both sides of the proboscis and are occasionally connected by a proboscidial plexus.

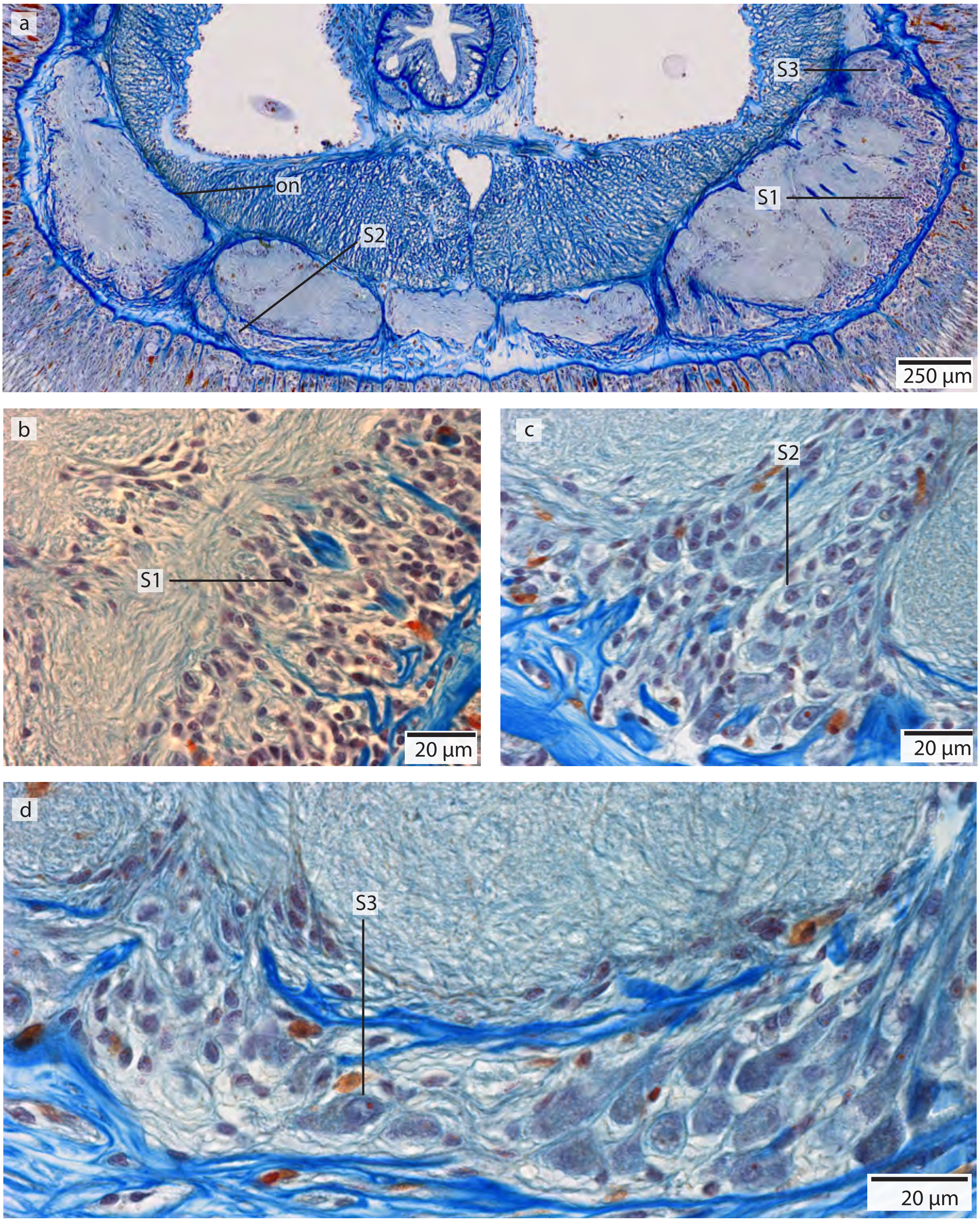


Fig. 15: *Tubulanus superbus,* LM, cross sections, Azan. **a**: Overview of the brain showing the location of the different neuronal cell somata (*S1*-*S3*) of the brain. The brain is surroundded by a prominent outer neurilemma (*on*). **b**: The nuclei of neuronal cell somata type *1* dye *purple*. The perikarya are not enlarged. **c**: Nuclei of neuronal cell somata type *2* dye *orange*. The perikarya are enlarged. **d**: Cell bodies of type *3* neuronal cell somata are very prominent. The nuclei dye *orange*.

Sensory structures

Cerebral organ

The cerebral sense organs are located in the posterior part of the brain on the level where the dorsal and ventral lobes of the brain divide (fig. 14c). The epidermal canals of the cerebral sense organs open laterally to the exterior (fig. 16a). Each canal is a small ciliated tube which runs towards the inner of the animal and ends blindly (fig. 16c). The canal terminates in exterior to the basal lamina of the epidermis. The neuronal cell somata close to the canal are of the type 3 described for the brain (fig. 16b). The cerebral sense organ is connected to the dorsal lobe of the brain by small branches of neurites; there is contact to the blood vessels.

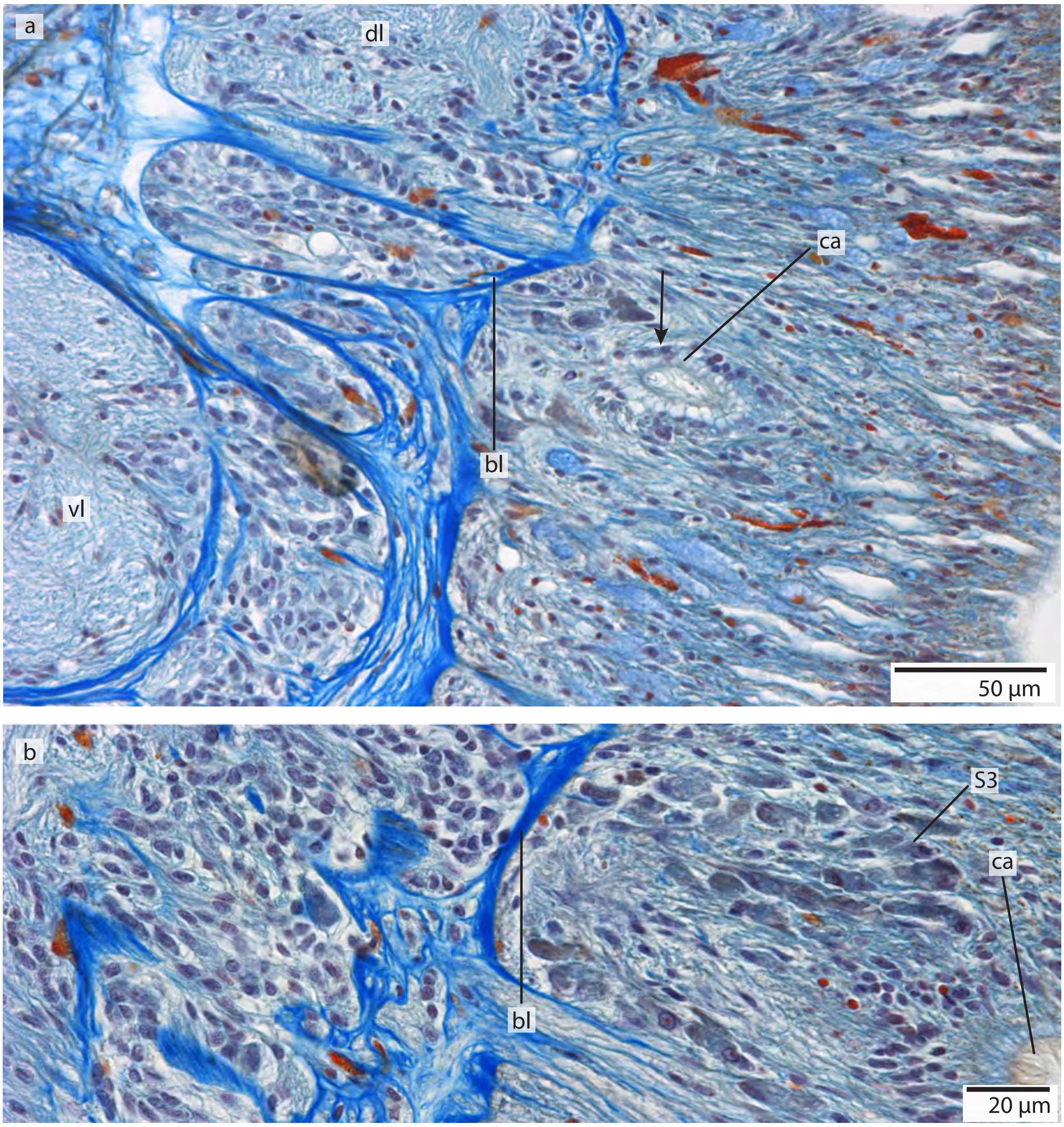


Fig. 16: *Tubulanus superbus,* LM, cross sections, Azan. **a**: The sensory cells (*arrow*) surrounding the canal (*ca*) of the cerebral organ are connected to the dorsal lobe (*dl*) of the brain. The canal ends anterior to the basal lamina (*bl*) of the epidermis. *vl*: ventral lobe. **b**: The cells adjacent to the cerebral organ are of type 1 described for the brain.

3.1.4 *Tubulanus polymorphus* (Tubulanidae, "Palaeonemertea")

The brain of *Tubulanus polymorphus* is situated closely anterior to the mouth opening. It is embedded underneath the basal lamina of the epidermis (subepidermal) and is not externally visible (fig. 17a).

Brain and central nervous system

The posterior part of the brain is divided into a ventral and dorsal lobe, on the level where the duct of the cerebral sense organ gains contact to the environment. The two parts of the brain are connected by commissural tracts (fig. 17b). The dorsal parts of the brain are connected by several small dorsal commissural tracts. Whether these tracts are independent from each other or fused, is not clearly discernable (fig. 17b). The ventral commissural tract is more prominent and is situated posterior to the dorsal ones (fig. 18b). The ventral lobe is confluent with the lateral medullary cords (fig. 18c), while the dorsal lobe ends blindly. The lateral medullary cords are embedded between the basal lamina of the epidermis and small layer of circular muscle (fig. 18d). The neuronal cell somata of the medullary cords cover the neuropil dorsally and ventrally (fig. 18d). The neuronal cell somata here are of the 1 type described for the brain. The neurites of the neuropil of the medullary cords branch into the neuronal cell somata.

The brain of *T. polymorphus* is composed of a central of neuropil which is surrounded by a layer of neuronal cell somata (figs. 17d; 18b; 19a- c). There are at least three types of neuronal cell somata discernable (fig. 19a- c). The nuclei of neuronal cell somata type 1 stain dark purple, cell bodies are small, elongated and they appear in cluster (fig. 19b). The nuclei of type 2 neuronal cell somata stain orange and cell bodies have a circular appearance (fig. 19b). These cells are only found in small numbers. The neuronal cell somata are evenly distributed all over the brain. Cell bodies of type 3 neuronal cell somata are enlarged and nuclei stain red (fig. 19c). The neuronal cell somata are not separated by an inner extracellular matrix (*ecm*, inner neurilemma) to the neuropil. A prominent outer *ecm* (outer neurilemma) surrounds the whole brain.

Minor nerves and peripheral nervous system

The numerous cephalic nerves are embedded between the basal lamina of the epidermis and a layer of longitudinal muscles. The nerves are arranged circularly around the margins of the head (figs. 17b; 18a) and separated by a neurilemma from each other.

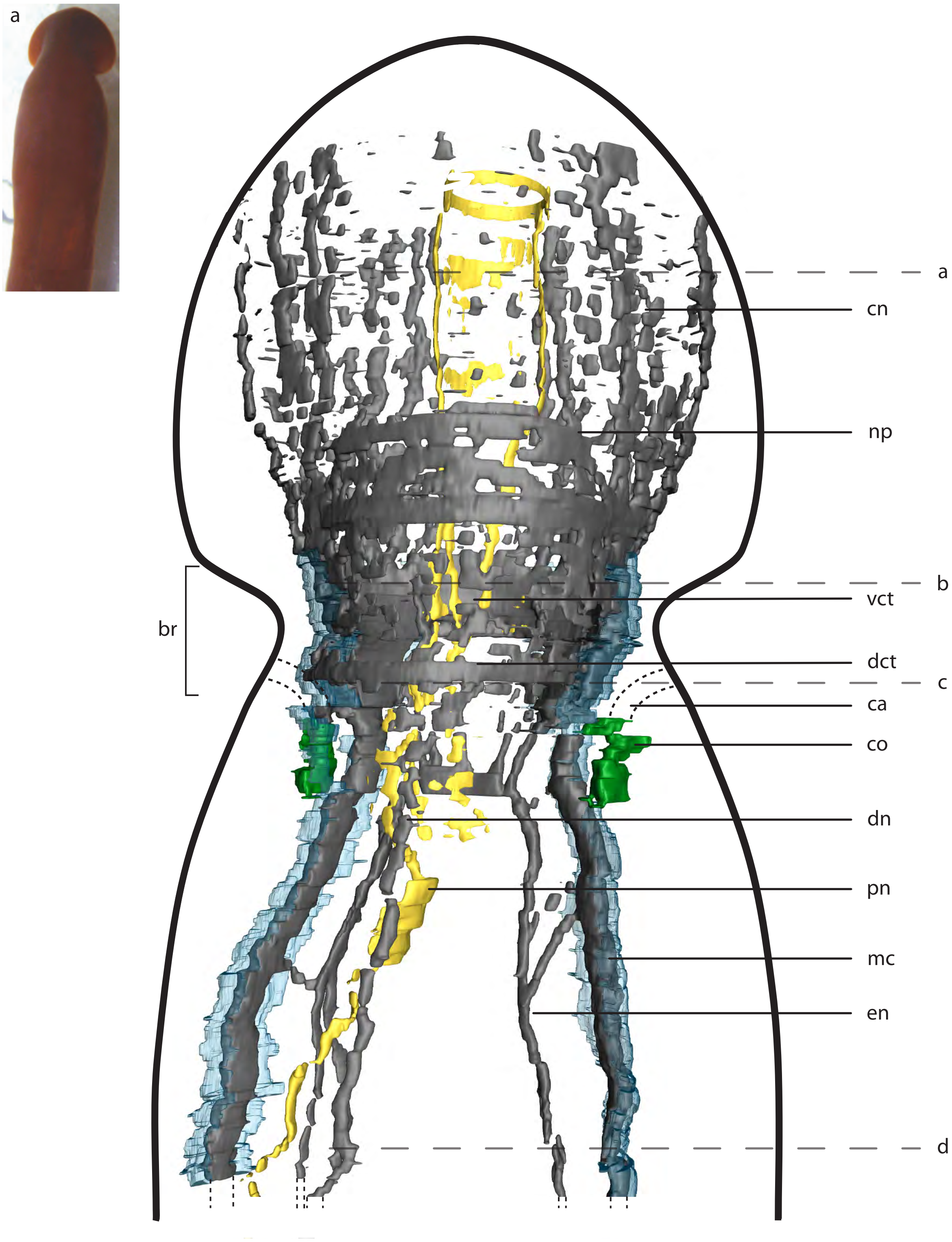


Fig. 17: *Tubulanus polymorphus*. **a**: Living specimen. **b**: Central nervous system, dorsal view, snapshot 3d-reconstruction (173 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are circular arranged around the animals´head, the paired proboscidial (*pn*, *yellow*) nerves originate from the ventral commissural tract (*vct*). A dorsal nerve (*dn*) originates from the dorsal commissural tract (*dct*). The cerebral organs (*co*, *green*) gain contact to the environment via small canals (*ca*) which open lateral. The cerebral organ is connected to the dorsal lobe of the brain. The branching esophageal nerves (*en*) originate from the ventral commissural tract. The lateral medullary cords (*mc*) originate in the ventral lobes of the brain. letters on the right (**a** - **d**) refer to the histological sections in figure 18.

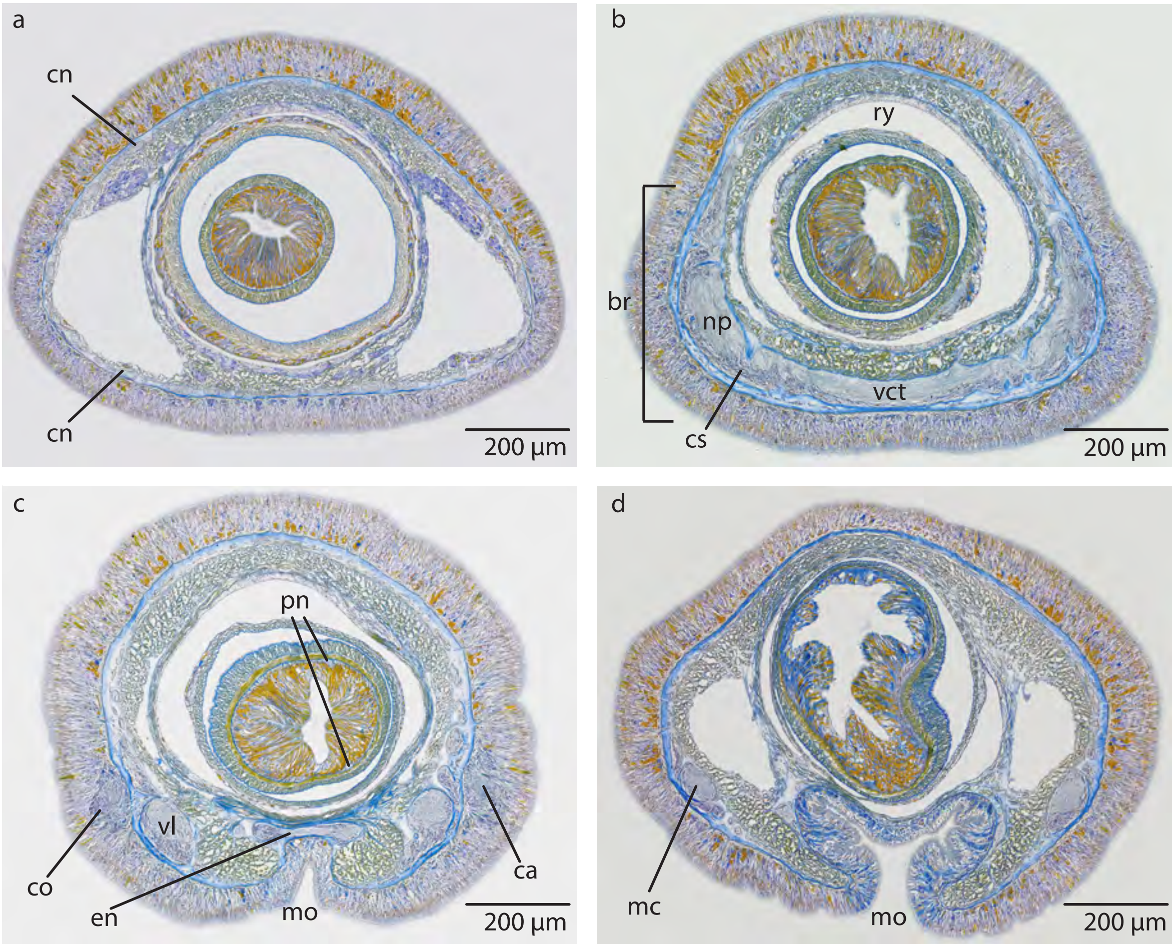


Fig. 18: *Tubulanus polymorphus,* LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are circular arranged around the tip of the head. **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*), the ventral commissural tract (*vct*) connects the two halfes of the brain below the rhynchocoel (*ry*). **c**: The canals (*ca*) of the cerebral organ (*co*) open ventro- laterally to the environment. The esophageal nerves (en) originate in the ventral commissural tract and are connectd again on the level of the mouth opening (*mo*). Two proboscidial nerves (*pn*) are present. *vl*: ventral lobes of brain. **d**: The ventral lobes of the brain are confluent with the lateral medullary cords. *mo*: mouth opening.

The cephalic nerves are not covered by cell somata.

A dorsal nerve arises from the posterior dorsal commissural tract and runs through the animal underneath the basal lamina of the epidermis (subepidermal) (fig. 18c).

The esophageal nerves arise posterior to the ventral commissural tract from the ventral lobe of the brain. The nerves are joined anterior to the mouth opening before they branch again and run along the lateral part of the foregut (fig. 18c).

The proboscis nerves arise from the ventral commissural tract (fig. 18c). The nerves run opposed to each other along both sides of the proboscis and are connected by a proboscidial plexus.

Immunostaining reveals several nerve plexus in *T. polymorphus* (figs. 20a- d). The neurites of the intraepidermal nerve plexus form a regular, ladder-like meshwork (fig. 20b). The neurites of the subepidermal plexus show immunoreactivity against FMRF (fig. 20a). The commissural plexus is well developed (fig. 20d) and connects the two medullary cords. The neurites of the latter two plexus are horizontally arranged. The neurites of the intrastomatogastric nerve plexus are arranged in an irregular net-like manner (fig. 20c).

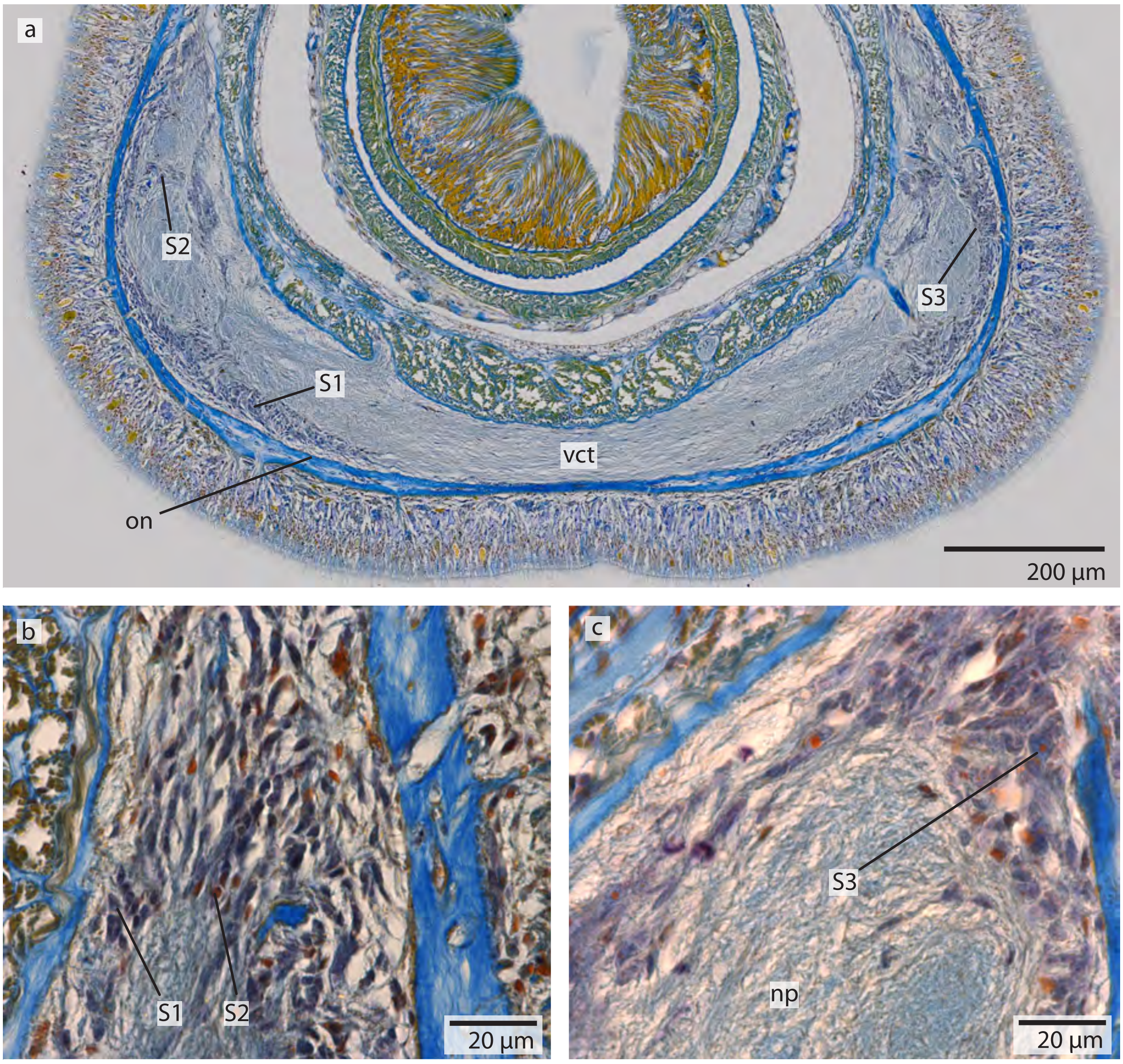


Fig. 19: *Tubulanus polymorphus,* LM, cross sections, Azan. **a**: Overview of the brain region showing the location of the different cell somata types 1- 3 (*S1*- *S3*). The brain is surroundded by a prominent outer neurilemma (*on*). *vct*: ventral commissural tract. **b**: Higher magnifcation of *S1*, *S2*. Nuclei of neuronal cell somata type 1 (*S1*) stain *purple*. The cell body is not enlarged. Nuclei of *S2* stain *red*, *S2* are solitary distributed all over the brain. **c**: Nuclei of neuronal cell somata type *3* stain *red* and the cell body is more prominent compared to *S1* and *S2*. *np*: neuropil.

Sensory structures

Cerebral organ

The cerebral sense organs are located in the very posterior part of the brain. Each epidermal canal of the cerebral sense organs open laterally to the exterior (figs. 17b; 18d). The canal is a small ciliated tube which runs towards the inner of the animal but does terminate anterior to the basal lamina of the epidermis. The sensory cells lining the canal are connected to the ventral lobe of the brain by small branches of neurites (fig. 18c). The neuronal cell somata surrounding the duct are of type 1 described for the brain.

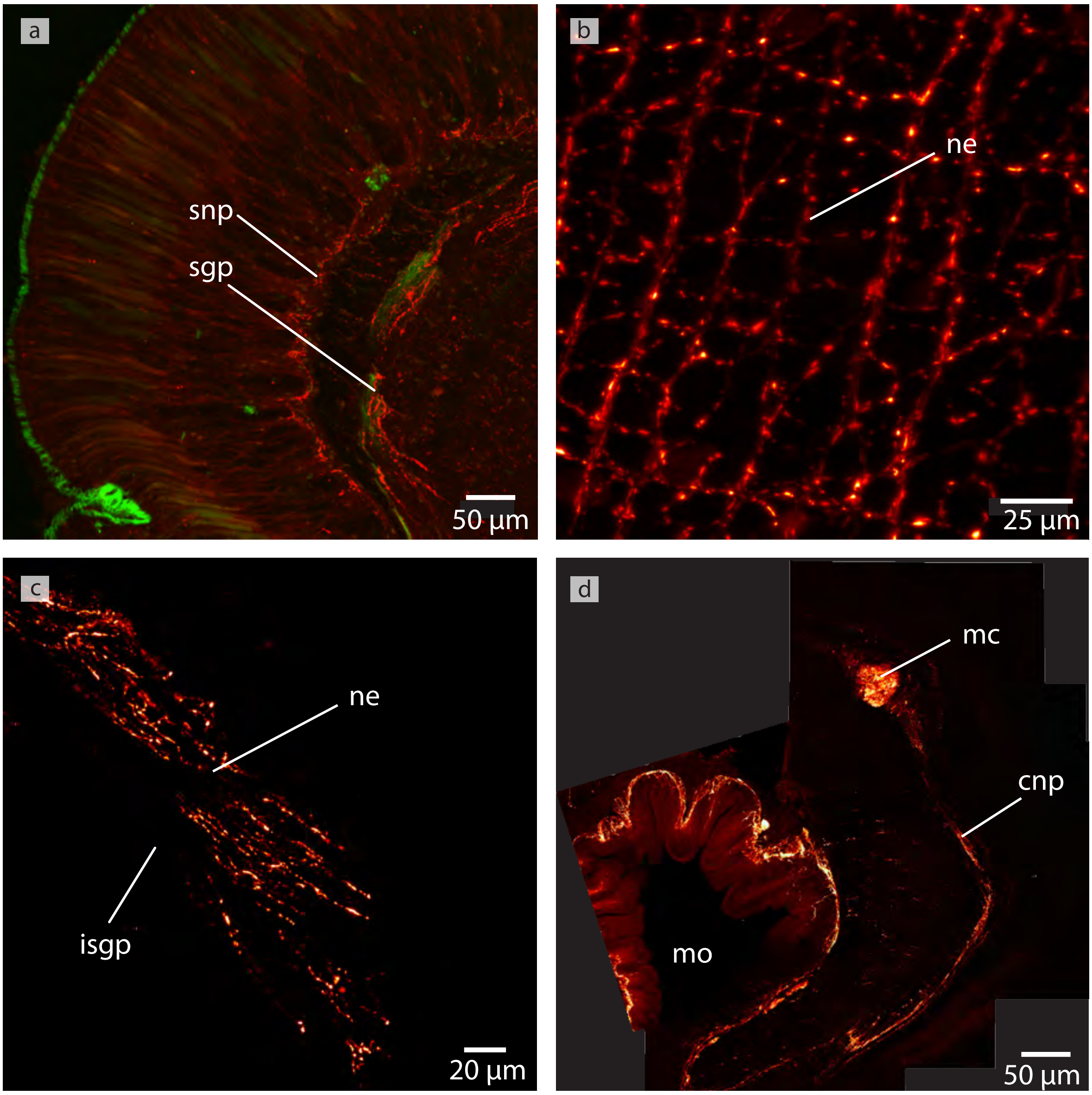


Fig. 20: *Tubulanus polymorphus,* CLSM **a**: Cross section, anti- FMRF (*red*), anti Tubulin (*green*) showing the subepidermal (*snp*) and the stomatogastric (*sgp*) nerve plexus. **b**: Horizontal section, anti FMRF showing the regular arranged neurites (*ne*) of the subepidermal nerve plexus. **c**: Horizontal section, anti FMRF. The neurites (*ne*) of the intrastomatogastric nerve plexus (*isgp*) are arranged in a regular ladder-like fashion. **d**: Cross section, anti Serotonin. The lateral medullary cords (*mc*) are interconnected by a commissural plexus (*cnp*). The commissural plexus surrounds the mouth opening (*mo*).

3.1.5 *Carinina ochracea* (Tubulanidae, “Palaeonemertea”)

The brain of *Carinina ochracea* is located close to the mouth opening. It is embedded above the basal lamina of the epidermis (basiepidermal) and externally visible (fig. 21a,b).

Brain and central nervous system

The very posterior part of the brain is divided into a dorsal and ventral lobe on the level where the duct of the cerebral sense organ gains contact to the environment (figs. 22b, c). Both lobes of the brain are interconnected by commissural tracts. The ventral commissural tract is anterior to the dorsal one and more prominent than the latter (figs. 22b, 24b). The dorsal lobe ends blindly in a layer of cell somata. The ventral lobes taper posteriorly and give rise to the lateral medullary cords. The lateral medullary cords run to the posterior above the basal lamina of the epidermis (basiepidermal) (fig. 22d). The neuronal cell somata cover the neuropil of the medullary cords dorsally and ventrally. The somata are of type 2 described for the brain (fig. 22d).

The brain is composed of a central neuropil which is surrounded by neuronal cell somata (figs. 21b; 22b; 24a- d). Two types of neuronal cell somata are discernable (fig. 23a). Cell bodies of type 1 neuronal cell somata are small and circular the nucleus stains dark purple. (fig. 23a). Cell bodies of type 2 neuronal cell somata are slightly enlarged and the nucleus also stains purple (figs. 23a, b). The different types of neuronal cell somata cannot be discriminated with immunostaining techniques (fig. 24a- d). Another type of neuronal cell somata is found around the terminal part of the canal of the cerebral sense organ (fig. 23c). The nuclei dye purple and the cell bodies are elongated. But wether these belong to the brain or the cerebral organ is not discernable.

The central part of the brain neuropil is separated by an inner neurilemma from the neuronal cell somata. An outer neurilemma is not or only partly present. Fibers of extracellular matrix branch into the brain neuropil (fig. 22d).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *C. ochracea* are in the very frontal part located dorsally and ventrally. The cephalic nerves originate from the lateral margins of the brain and are arranged circularly around the animals head (figs. 21b; 22a). The nerves are separated from each other by an extracellular matrix.

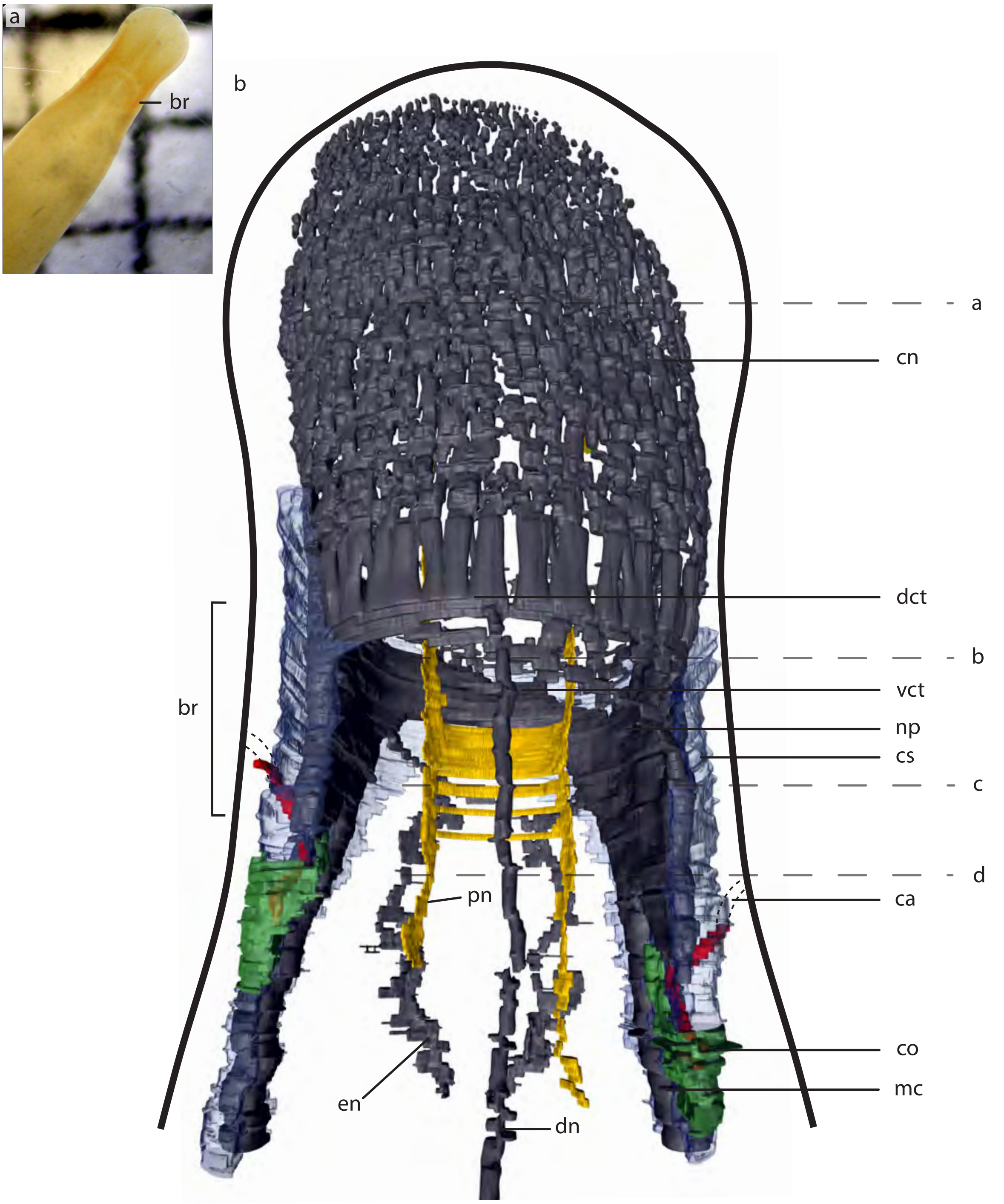


Fig. 21: *Carinina ochracea.* **a**: Living specimen (*br*: brain). **b**: central nervous system, dorsal view, snapshot 3d-reconstruction (160 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (cn) are circular arranged around the animal's head, the paired proboscidial nerves (*pn*, *yellow*) originate from the ventral commissural tract (*vct*). A dorsal nerve strand (*dn*) originates from the dorsal commissural tract (*dct*). The lateral medullary cords (*mc*) originate in the ventral lobe of the brain. The cerebral organs (*co*, *green*) gain contact to the environment via small cannals (*ca*, *red*) which open lateral. The cerebral organ is connected to the dorsal lobe of the brain. The branching esophageal nerves (*en*) originate from the ventral commissural tract (*vct*). The lateral medullary cords (*mc*) originate in the ventral lobes of the brain. letters on the right (**a** - **d**) refer to the histological sections in figure 22.

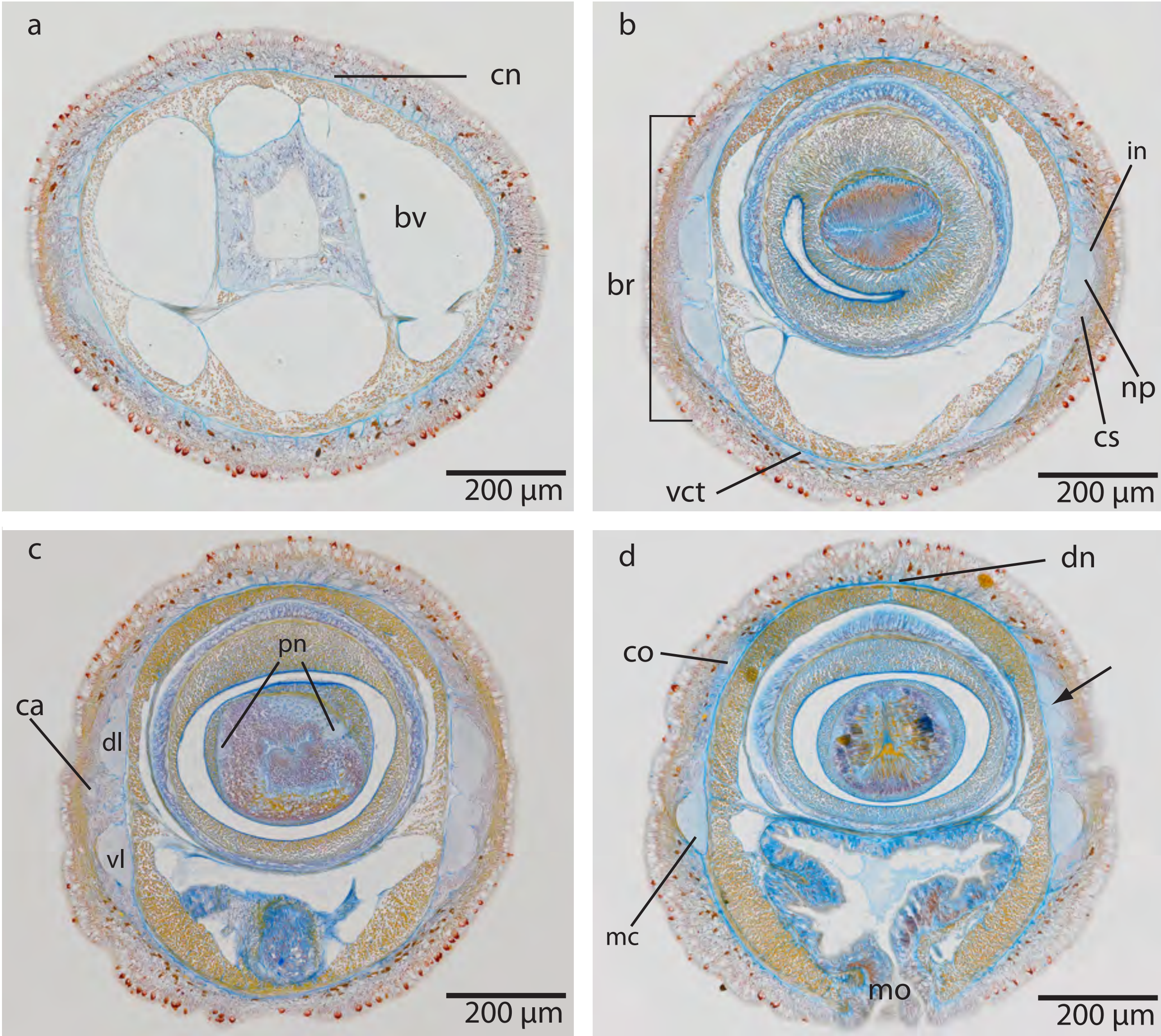


Fig. 22: *Carinina ochracea*, LM, cross sections, Azan. **a**: Frontal region showing the circular arranged cephalic nerves (*cn*). *bv*: blood vessel. **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*), a ventral commissural tract (*vct*) connects the two halfes of the brain, an outer neurilemma (*on*) encloses the whole brain. **c**: On the level of the division of the brain into a dorsal (*dl*) and ventral (*vl*) lobe the canals (*ca*) of the cerebral organs open lateral, two proboscidial nerves (*pn*) are present. **d**: The cerebral organs (*co*) end dorsally, a dorsal nerve strand (*dn*) arises from the dorsal commissural tract, the ventral part of the brain is confluent with the lateral medullary cords (*mc*). *note* the ecm (*arrow*) branching into the neuropil of the brain. *mo*: mouth opening.

A dorsal nerve arises from one of the dorsal commissural tracts and runs to the posterior directly above the basal lamina of the epidermis (basiepidermal) (fig. 22d).Another dorsally located nerve runs above the *ecm* of the rhynchocoel. A ventral nerve is not present.

The two esophageal nerves arise from the ventral lobes of the brain shortly posterior to the division into a dorsal and ventral lobe. They run ventrally and migrate inwards on the level of the mouth opening (fig. 21b). Then they run along both sides of the foregut. The two proboscis nerves arise from the ventral commissural tract (fig. 21b). The nerves run opposed to each other along both sides of the proboscis and are connected by a proboscidial plexus.

Several different nerve plexus are present in *C. ochracea* (figs. 25a- e). The neurites of the intraepidermal plexus are immunoreactive against FMRF-amid (fig. 25a) and form a ladder-like meshwork (fig. 25b). A subepidermal nerve plexus is visible in staining against FMRF-amid and α-tubulin (figs. 25a, c, d). This plexus gives rise to neurites which connects the plexus to the lateral medullary cords (figs. 25c, d). At the points where these neurites branch off, there is a neurite concentration of the subepidermal nerve plexus (fig. 25d), which appear spherical. The neurites of the intrastomatogastric nerve plexus are arranged in a defuse net-like manner (figs. 25e). A commissural and rhynchocoelan plexus was not discernable.

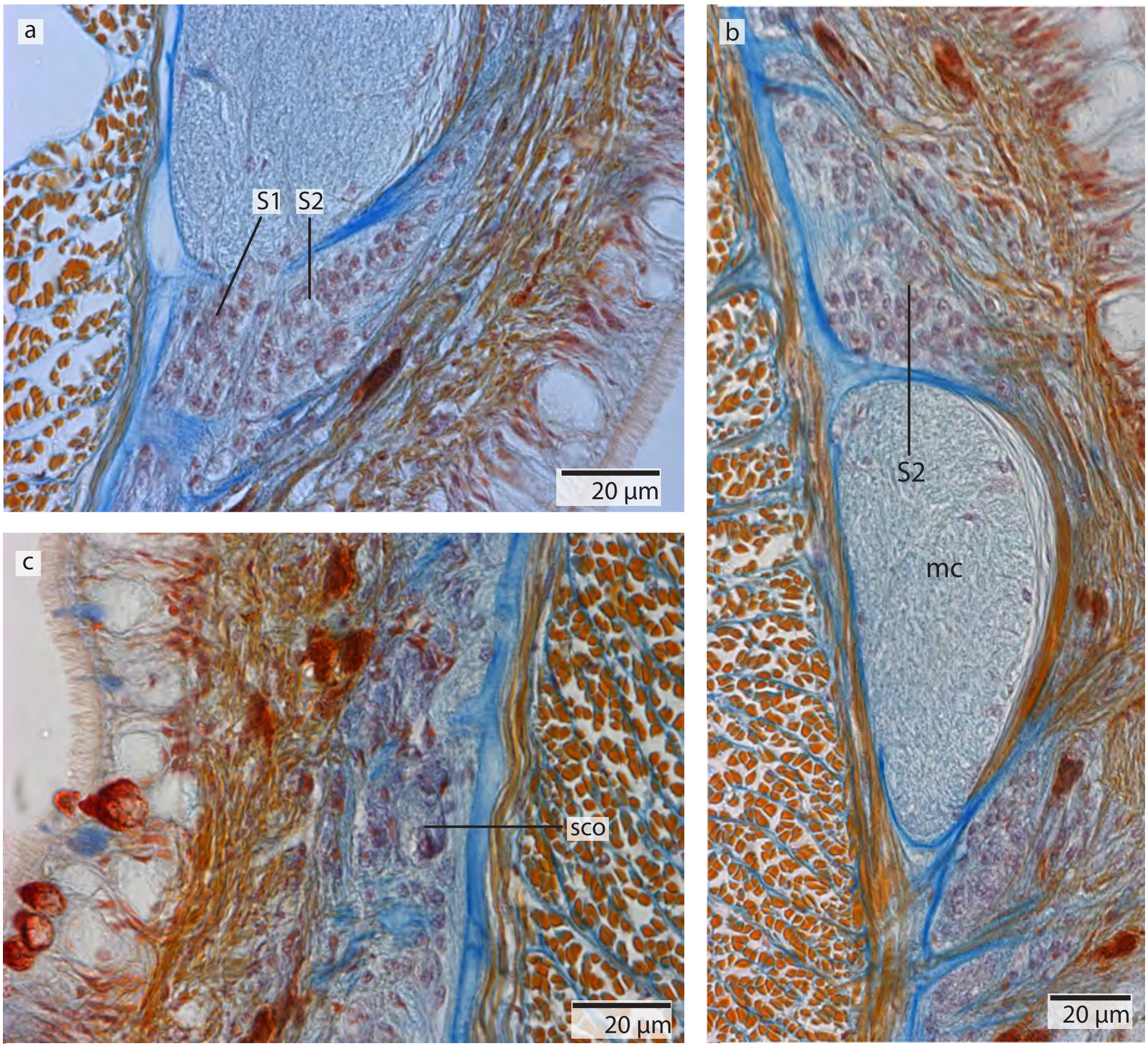


Fig. 23: *Carinina ochracea*, LM, cross sections, Azan. **a**: Cell bodies of neuronal cell somata type 1 (*S1*) are not enlarged and the nuclei stain *purple*. Cell bodies of neuronal cell somata type 2 (*S2*) are slightly enlarged and the nuclei also stain *purple*. **b**: Neuronal cell somata of the medullary cord (*mc*) are of the 2 type described for the brain. **c**: The canal of the cerebral organ ends in a layer of special neuronal cell somata (*sco*), which are not present in the brain.

Sensory structures

Cerebral organ

The cerebral sense organs are tube like structures which open lateral in a pit behind the division of the brain into a dorsal and ventral lobe (figs. 21b; 25a- c).

The epidermal canals are lined with sensory cells and enter the neuronal cell somata of the brain on the level of the division of the brain into a dorsal and ventral lobe (fig. 22c). Here the ciliated canal turns dorsal and runs along the margins of the basal lamina of the epidermis (fig. 22d).

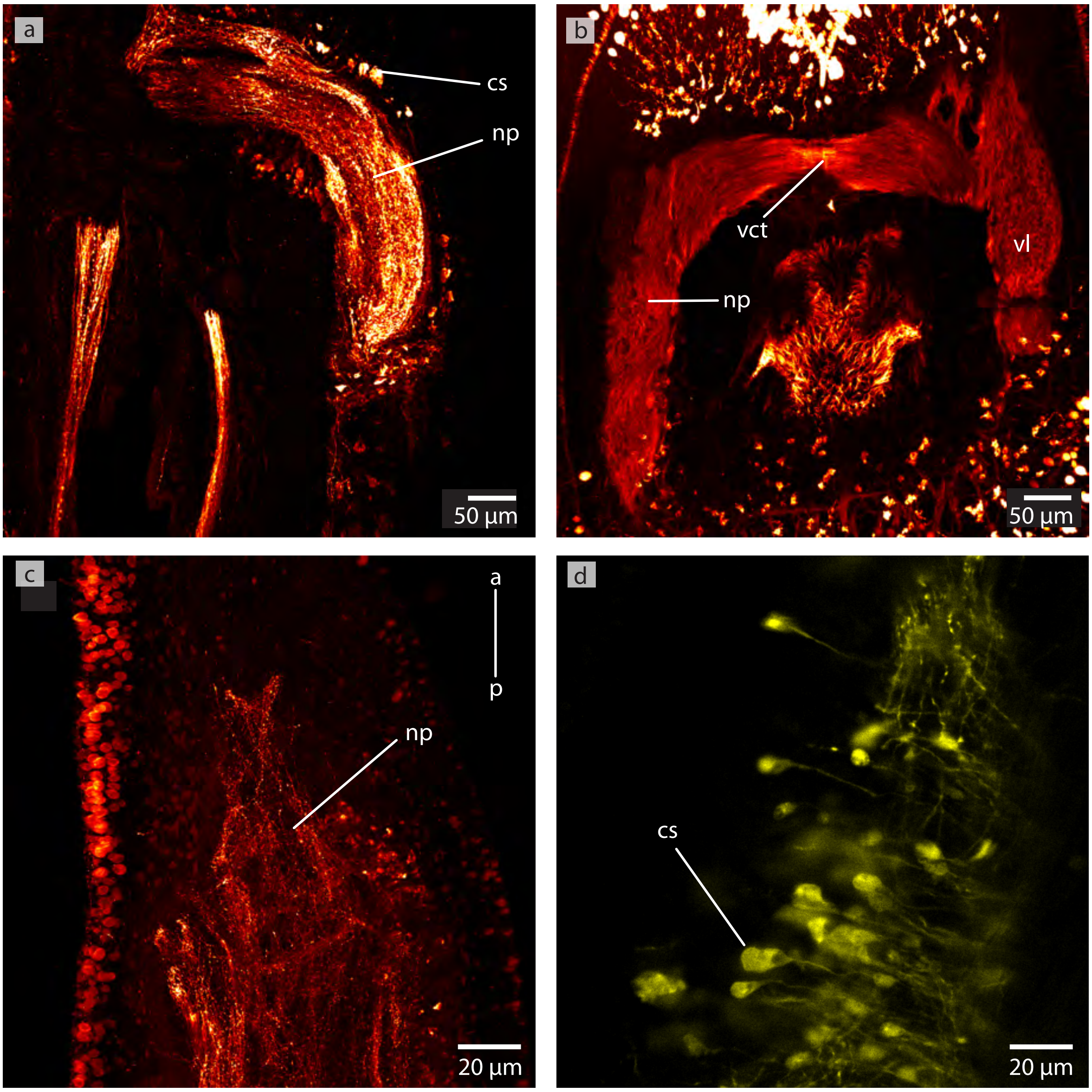
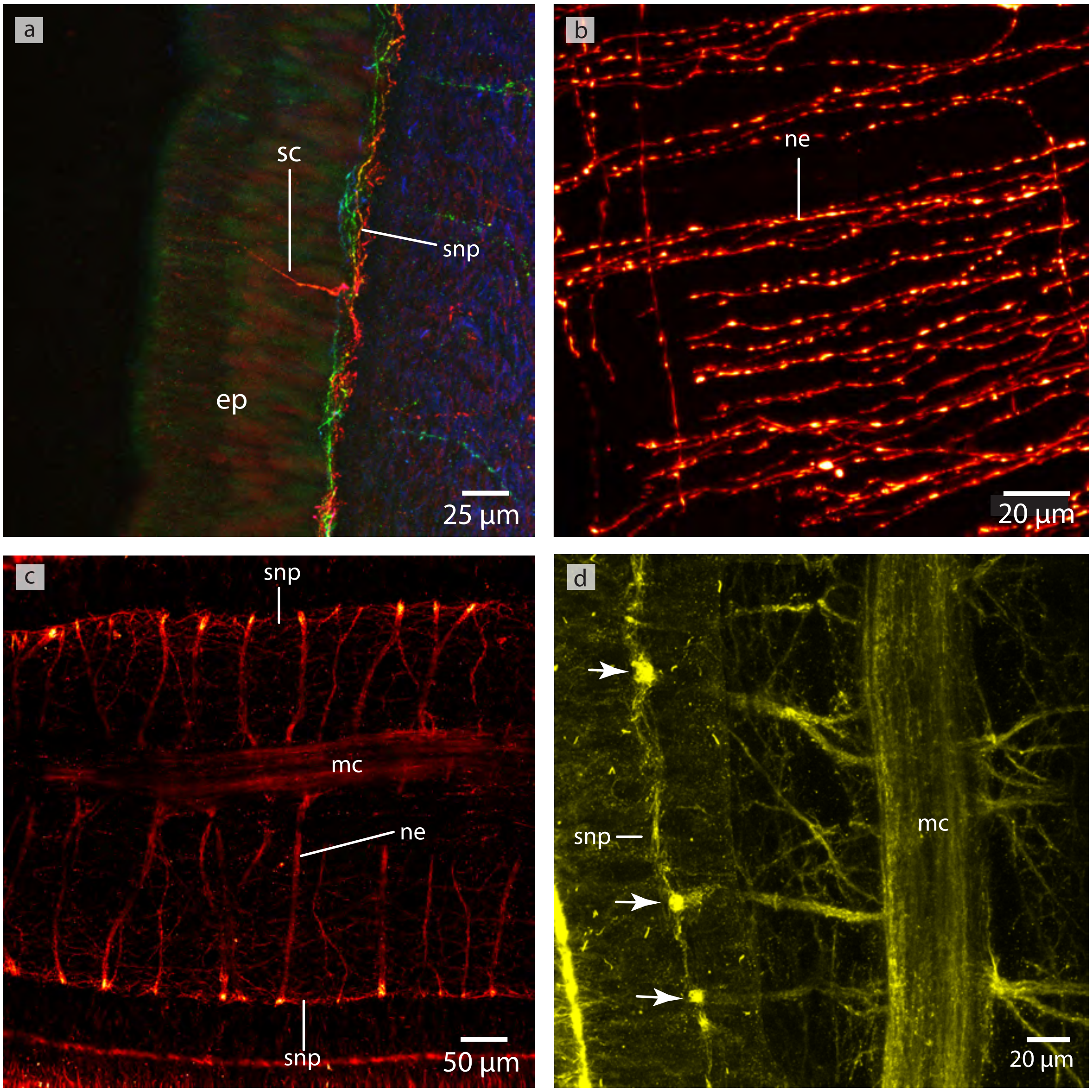


Fig. 24: *Carinina ochracea,* CLSM, horizontal sections. **a**: Anti serotonin, the brain is composed of neuropil (*np*) and cell somata (*cs*), note the few neuronal cell somata showing immunoreactivity against serotonin. **b**: Anti α-tubulin, the neurites of the neuropil (*np*) of the brain are horizontally arranged in the ventral commissural tract (*vct*). *vl*: ventral lobe. **c**: Anti serotonin, only few neurites show immunoreactivity against serotonin. *a*: anterior *np*: neuropil, *p*: posterior. **d**: Anti FMRF. The cell somata (*cs*) may represent glia cells.



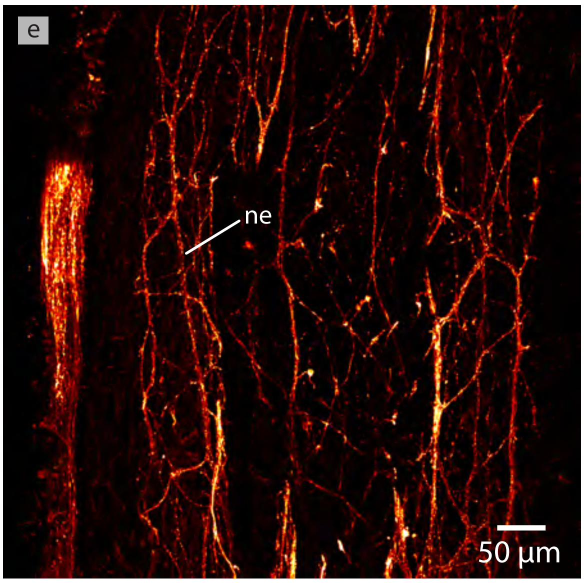


Fig. 25: *Carinina ochracea*, CLSM **a**: Anti FMRF, horizontal section. A sensory cell (*sc*) originates in the subepidermal plexus (*snp*) and proceeds into the epidermis (*ep*). **b**: Anti FMRF, horizontal section. The neurites (*ne*) of the intraepidermal nerve plexus are arranged in a ladder-like way. **c**: Anti α-tubulin, sagital section. The lateral medullary cords (*mc*) are interconnected to the subepidermal plexus (*snp*) by branches of neurites (*ne*). **d**: Higher magnification of picture **c**. *note* the concentration of neurites in the supepidermal nerve plexus (*arrows*) **d**: Anti FMRF, horizontal section. The neurites (*ne*) of the intrastomatogastric nerve plexus are irregular arranged.

The duct passes through the neuronal cell somata of the brain and ends blindly dorso-lateral. Here immunostaining reveals a very brightly staining structure which presumably represents densely arranged cilia (fig. 26b) and Azan staining reveals cell somata which are different to those in the brain and to those in the medullary cords (fig. 23c).

The nuclei dye purple and the cell bodies are elongated. The receptive cells are connected to the posterior part of the dorsal lobe of the brain by small branches of neurites showing immunoreactivity against α-tubulin (figs. 25a- c). There is no contact to the blood vessels.

Sensory cells

Many sensory cells are distributed all over the animal. These cells originate in the subepidermal nerve plexus and proceed into the epidermis (fig. 25a). The cells might have a tactile function.

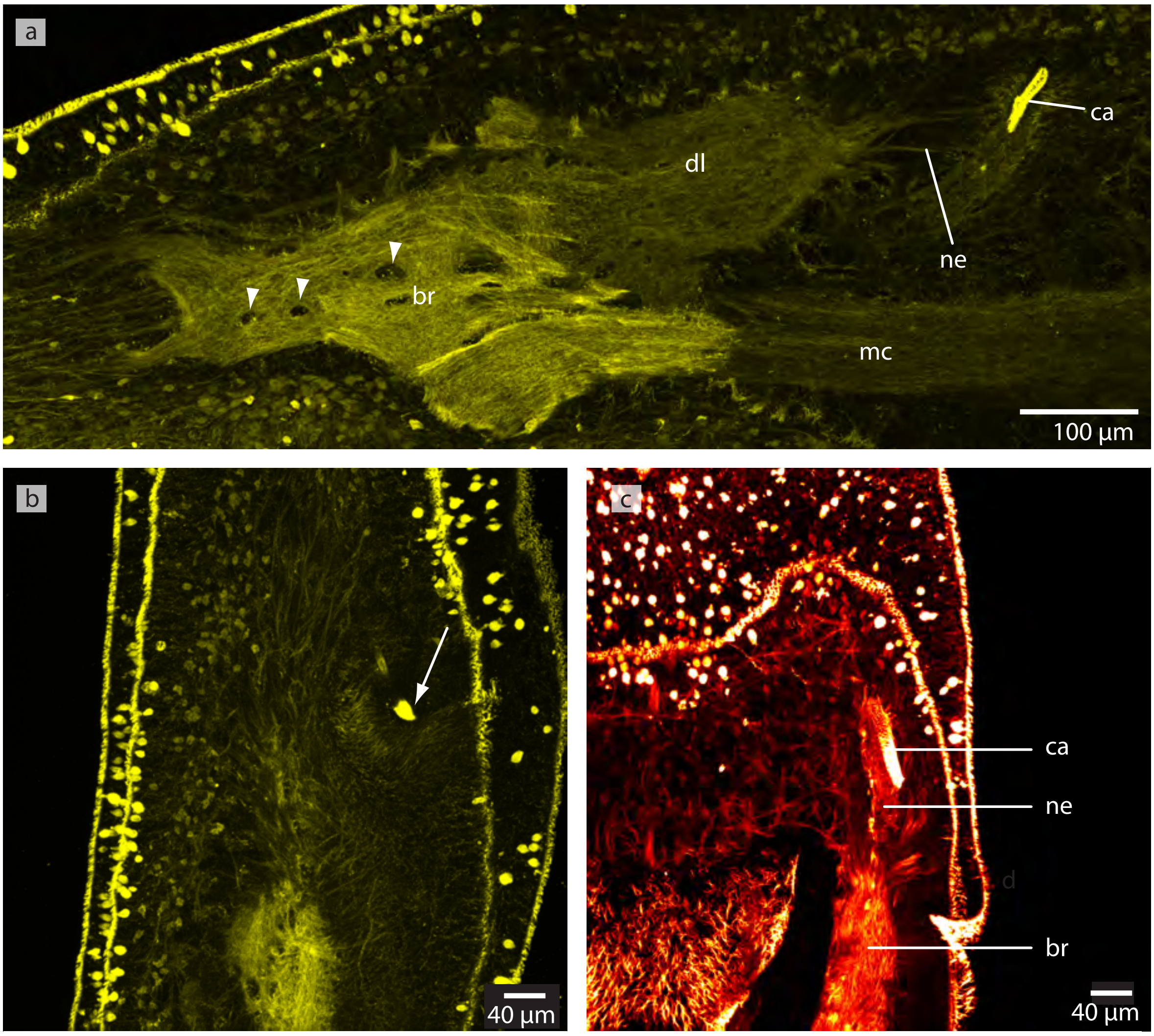


Fig. 26: CLSM, anti α-tubulin **a**: sagital section. The neurites (*ne*) of the cells lining the canal of the cerebral organ are connected to the neuropil of the dorsal lobe (*dl*) of the brain (*br*). Note the unstained structures in the neuropil of the brain (*arrowheads*). *mc*: medullary cord. **b**: sagital section. The canal terminates in a pit with a great number of cilia (*arrow*). **c**: horizontal section. The neurites (*ne*) of the sensory cells built a dense meshwork which is connected to the brain (*br*). *ca*: canal of the cerebral organ.

3.1.6 *Callinera monensis* (Tubulanidae, "Palaeonemertea")

The brain of *Callinera monensis* is located close to the mouth opening. It is embedded underneath the basal lamina of the epidermis (subepidermal).

Brain and central nervous system

The very posterior part of the brain is divided into a ventral and a dorsal lobe (fig. 28d). Both lobes are interconnected by commissural tracts (fig. 28c). The more prominent ventral commissural tract is on the same level with two independent small dorsal commissural tracts. The ventral lobe is confluent with the lateral medullary cords and the dorsal part ends blindly inside the neuronal cell somata of the brain. The lateral medullary cords lie directly underneath the basal lamina of the epidermis (subepidermal) (fig. 28f). Posterior to the brain the lateral medullary cords are connected by several small dorsal commissural tracts. The neuronal cell somata are arranged in a u-shaped manner around the neuropil of the medullary cords (fig. 28f). The somata cap the neuropil to the exterior.

The brain of *C. monensis* is composed of neuropil which is surrounded by a layer of neuronal cell somata (figs. 28b, c; 29a- c). There are three different types of neuronal cell somata discernable (fig. 29a- c). Cell bodies of type 1 neuronal cell somata are circular, not enlarged and the nucleus dyes purple (fig. 29b). These cells are primarily found on the dorsal lobe of the brain. Cell bodies of type 2 neuronal cell somata are pear shaped, the nuclei also stain purple (fig. 29c). These cells are found on the lateral and ventral parts of the brain. Cell bodies of type 3 neuronal cell somata are circular and much more prominent than the latter two; the nuclei also stain purple (fig. 29c). The brain neuropil is divided by an inner neurilemma from the cell somata. The whole brain is enclosed by an outer neurilemma (fig. 28d).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *C. monensis* consists of strands of neuropil and occasionally neuronal cell somata which are enclosed by a neurilemma. The cephalic nerves originate from the lateral margins of the head. The solitary distributed cell somata are not separated to the neuropil. The nerves are arranged circularly around the frontal part of the animal (fig. 27). These nerves are embedded between the basal lamina of the epidermis (subepidermal) and a small layer of circular muscles (fig. 28a).

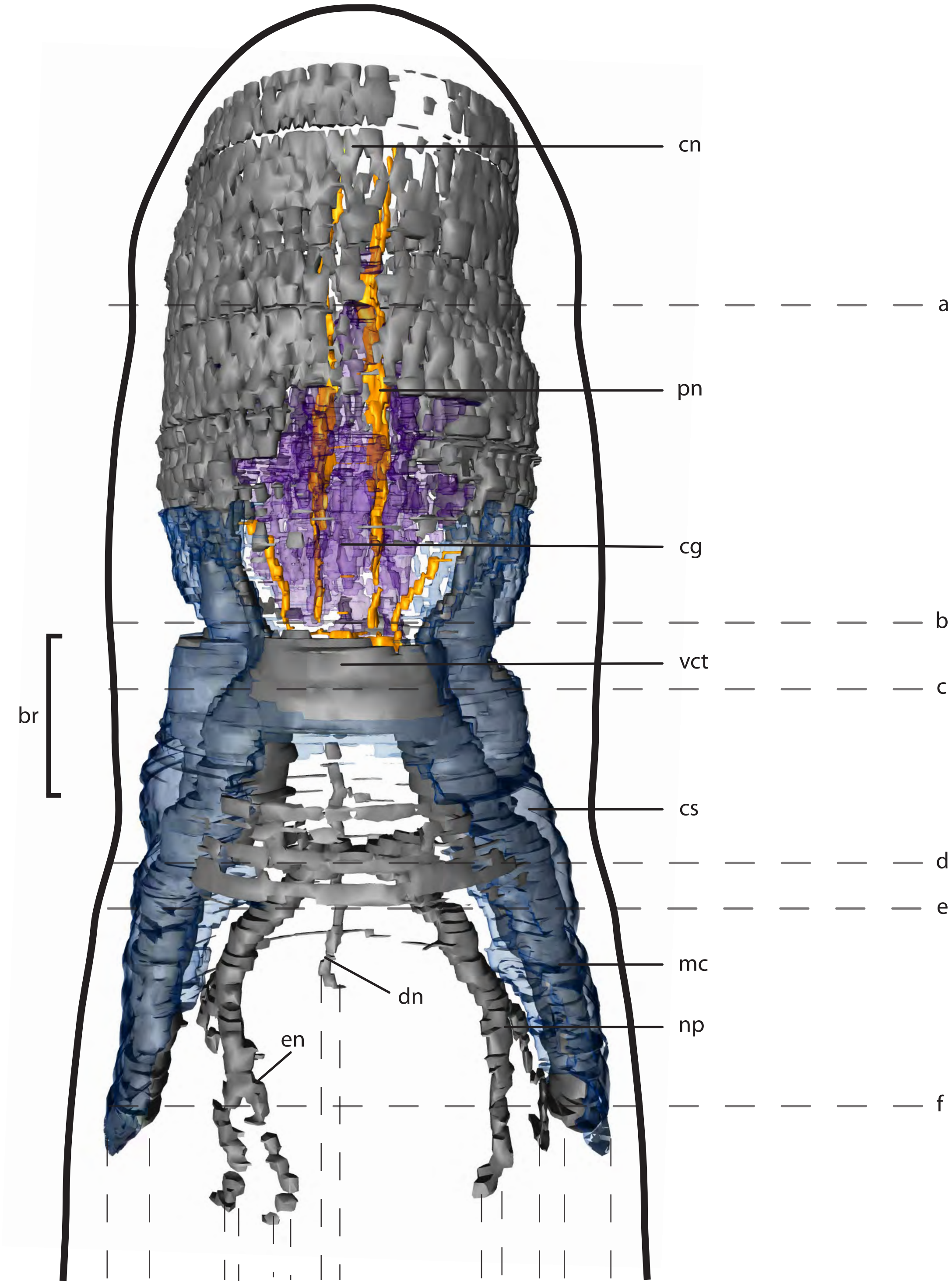


Fig. 27: *Callinera monensis* . Central nervous system, ventral view, snapshot 3d-reconstruction (140 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are circular arranged around the animal's head, the paired proboscidial nerves (*pn*, *yellow*) originate from the ventral commissural tract (*vct*). A cerebral gland (*cg*, *purple*) is dorsally located in the region anterior to the brain (*br*). A dorsal nerve strand (*dn*) originates from the dorsal commissural tract. The branching esophageal nerves (*en*) originate from the ventral commissural tract. The lateral medullary cords (*mc*) originate in the ventral lobes of the brain. letters on the right (a - f) refer to the histological sections in figure 28.

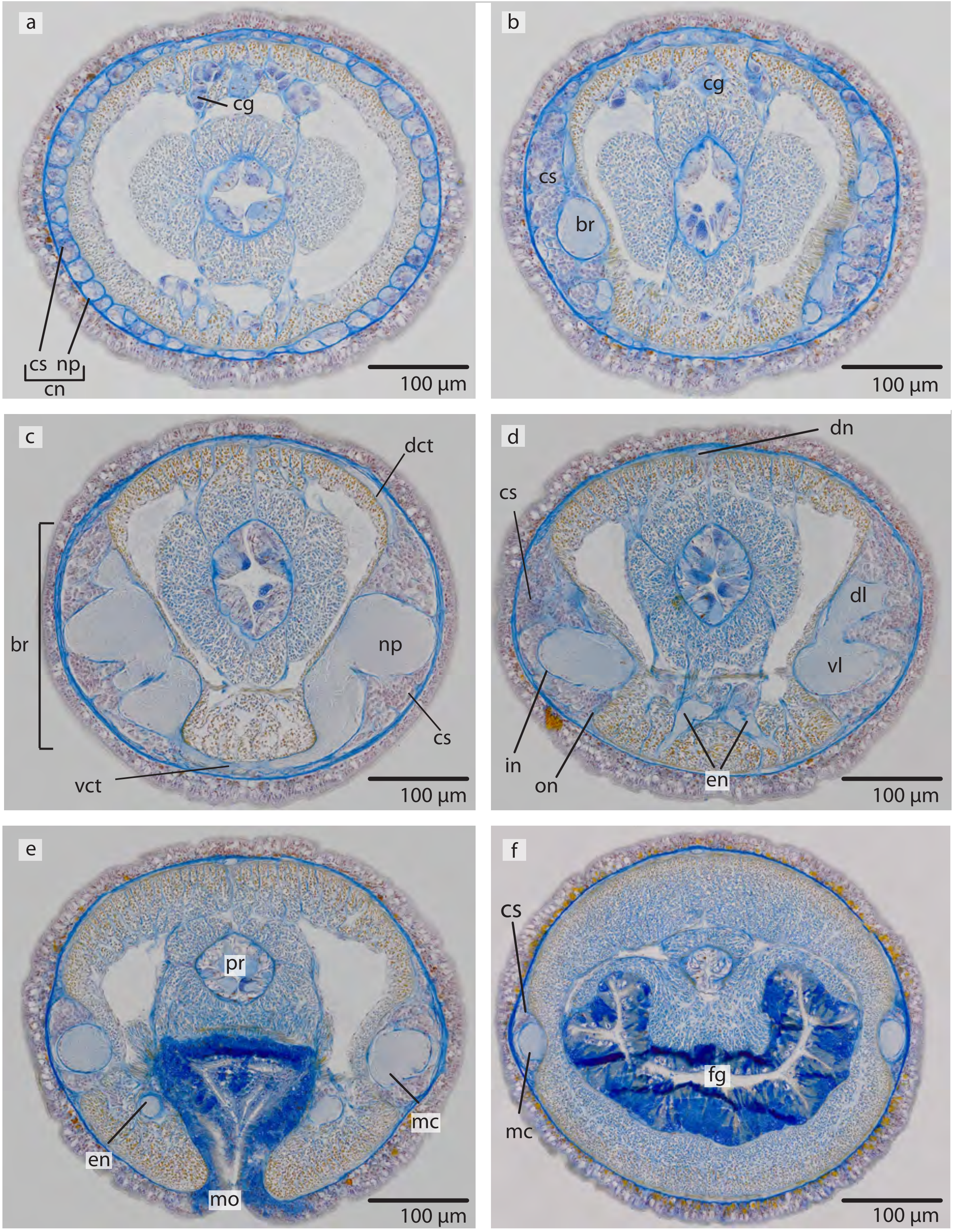


Fig. 28: *Callinera monensis*, LM, cross sections, Azan. **a**: The cepalic nerves (*cn*) are composed of neuropil (*np*) and neuronal cell somata (*cs*), a cephalic gland (*cg*) is located dorsally. **b**: The anterior region of the brain (*br*) is covered by a enormous layer of cell somata (*cs*). **c**: The brain is composed of a central neuropil (*np*) and a surrounding layer of cell somata (*cs*). The two halves of the brain are connected by a ventral (*vct*) and dorsal (*dct*) commissural tract. **d**: More posterior the brain is divided into a ventral (*vn*) and dorsal (*dl*) lobe, a dorsal nerve (*dn*) strand arises from the dorsal commissural tract, the paired esophageal nerves (*en*) arise from the ventral commissural tract. The somata are separated from the neuropil by an inner neurilemma (*in*), the whole brain is enclosed by an outer neurilemma (*on*). **e**: The ventral lobes of the brain are confluent with the lateral medullary cords (*mc*). *mo*: mouth opening, *pr*: proboscis. **f**: The lateral medullary cords (*mc*) run to the posterior of the animal. The cell somata (*cs*) cover the neuropil in a u- shaped manner. *fg*: foregut.

A dorsal nerve arises from the first dorsal commissural tract of the brain. It runs to the posterior embedded between the basal lamina of the epidermis and a layer of circular muscles (fig. 28d). A ventral nerve is not present.

Behind the brain the dorsal nerve is connected to the lateral medullary cords by several small commissural tracts. In front of the mouth opening, the posterior ventral part of the brain is connected by three small ventral commissural tracts.

The esophageal nerves arise from one of the posterior lying ventral commissural tracts behind the brain and fuse again in front of the mouth opening (figs. 27, 28e). Then the nerves divide again to run posterior on the lateral side of the mouth opening. On the level with the esophagus the nerves are bifurcated. Thus there are two dorso-lateral and two ventro-lateral esophageal nerves. The proboscidial nerves are connected to the central nervous system short posterior to the ventral commissural tract of the brain.

Sensory structures

No sensory structures such as eyes or a cerebral sense organ are present in *C. monensis*.

Cephalic glands

An enormous layer of cephalic glands are present in the frontal part of the animal anterior to the brain (fig. 28a- b). These might serve as neurosecretory structures as they are in close contact to the blood lacunae and have a connection to the dorsally located cephalic nerves.

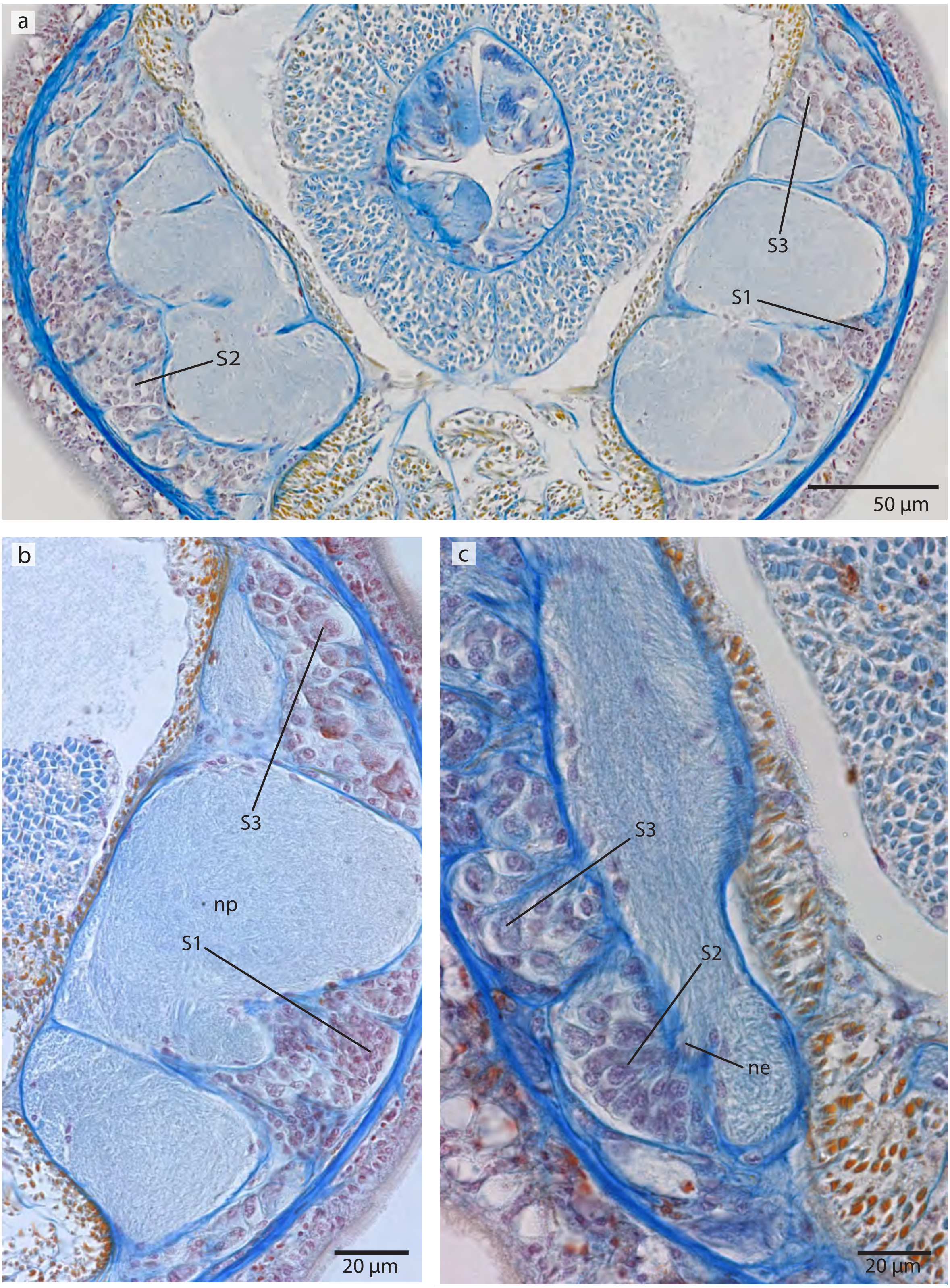


Fig. 29: *Callinera monensis*, LM, cross section, Azan. **a**: Overview of the brain region showing the location of the different neuronal cell somata types 1- 3 (*S1*- *S3*), all nuclei of the different cell somata dye *purple*. **b**: Higher magnifcation of cell somata 1, 3 (*S1*, *S3*). The cell bodies of *S1* are circular and not enlarged and they appear in cluster. The cell bodies of *S3* are also circular but much more prominent than in the other two cell somata types. *np*: neuropil. **c**: Higher magnifcation of cell somata *S2*, *S3*. Cell bodies of *S2* are pear shaped and show a slightly enlargement. The neurites (*ne*) of the brain neuropil branch into the cell somata layer.

3.1.7 ***Carinoma mutabilis*** (Carinomidae, "Palaeonemertea")

The brain of *Carinoma mutabilis* is situated closely anterior to the mouth opening. It is embedded in the head musculature. The brain is not externally visible (fig. 30a).

Brain and central nervous system

The posterior part of the brain is divided into a dorsal and ventral lobe. Both halves of the brain are connected by commissural tracts. The dorsal one appears prior to the ventral one which is more prominent (fig. 31b). The dorsal lobe is very short and ends blindly in a layer of neuronal cell somata. The dorsal lobe is connected by a nerve to the dorsal commissural tract of the brain (fig. 30b). The ventral lobe is confluent with the lateral medullary cords. The medullary cords run to the posterior of the animal between the outer longitudinal muscle layer and the inner circular muscle layer (fig. 31d). The neuronal cell somata of the medullary cords are separated by an inner neurilemma from the neuropil and are located dorsally and ventrally to the neuropil. The neuronal cell somata resemble the type 1 and 2 described for the brain (fig. 31d).

The brain of *Carinoma mutabilis* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata(figs. 30b; 31b, c; 32a- c). There are at least two types of neuronal cell somata discernable (figs. 32a, b). Nuclei of neuronal cell somata type 1 dye red and the cell bodies show no enlargement (figs. 32b). These cells are distributed all over the brain and are by far the most numerous (figs. 32a). Nuclei of neuronal cell somata type 2 dye purple (figs. 32b). The cell body of type 2 cell somata are slightly enlarged.

In the dorsal and ventral tips of the brain enormous cells are found. Nuclei of these cells dye red (figs. 32a, c). The cell body is very prominent and the cytoplasm dye bright purple (fig. 32c). These cells are only found on the dorsal and ventral tips of the brain posterior to the dorsal or ventral commissural tract respectively. This cell layer decreases to the posterior and disappears when the ventral lobes of the brain taper to the lateral medullary cords. On the dorsal tips of the brain the cells are in close contact to the blood vessels and may represent glandular cells and not neuronal cell somata (figs. 32a, c).

The neuronal cell somata are not separated by an inner neurilemma from the neuropil. The whole brain is surrounded by an outer neurilemma (fig. 31b).

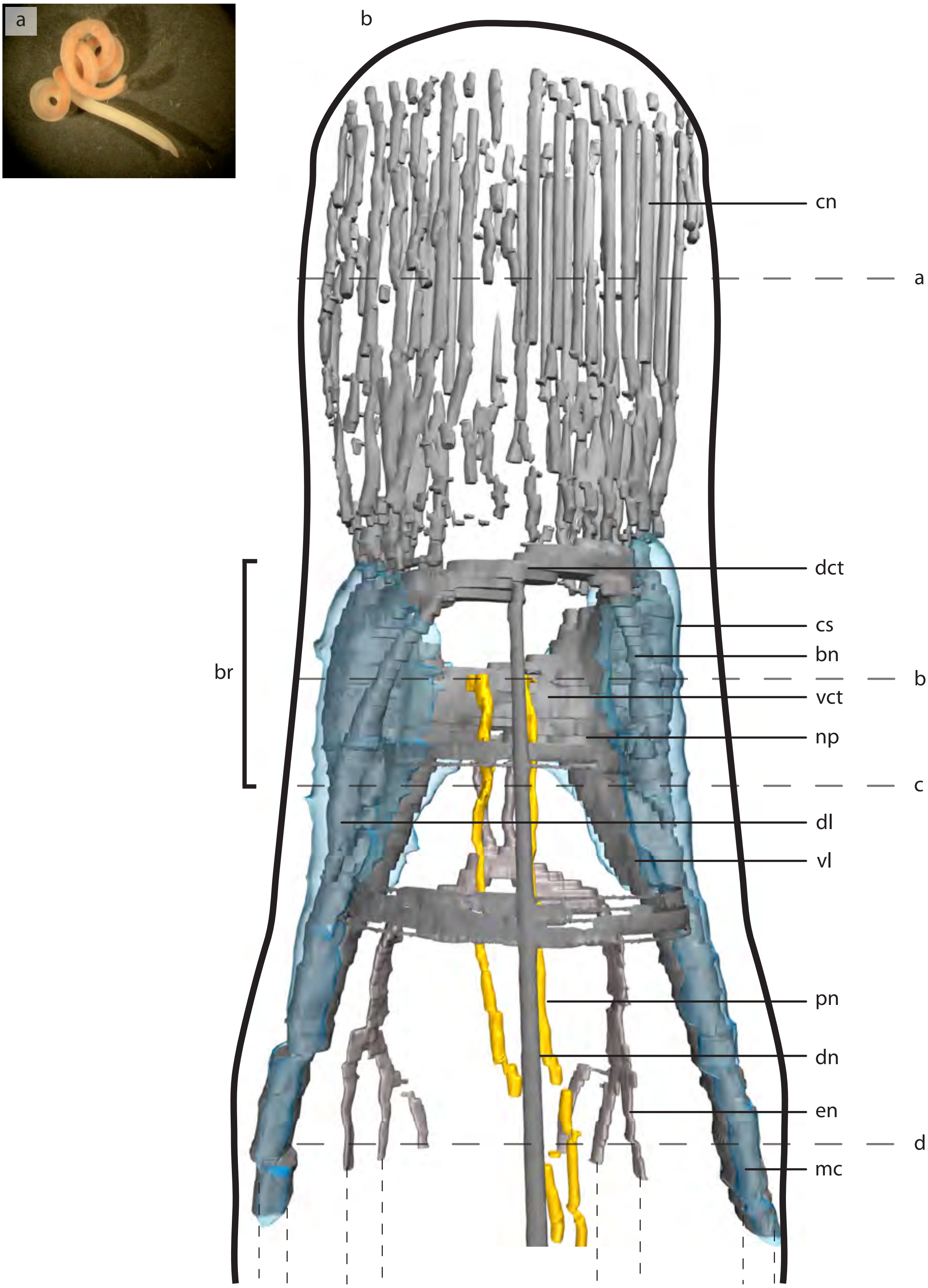


Fig. 30: *Carinoma mutabilis.* **a**: Living specimen from nemertea.lifedesks.org. **b**: Central nervous system dorsal view, snapshot 3d-reconstruction (137 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are circular arranged around the animal's head, the paired proboscidial nerves (*pn*, *yellow*) originate from the ventral commissural tract (*vct*). The posterior part of the brain is divided into a dorsal (*dl*) and ventral lobe (*vl*). A brain nerve (*bn*) connects the posterior part of the dorsal lobe with the dorsal commissural tract.A dorsal nerve strand (*dn*) originates from the dorsal commissural tract (*dct*). The branching esophageal nerves (*en*) originate from the ventral commissural tract. The lateral medullary cords (*mc*) originate in the ventral lobes of the brain. letters on the right (**a** - **d**) refer to the histological sections in figure 31.

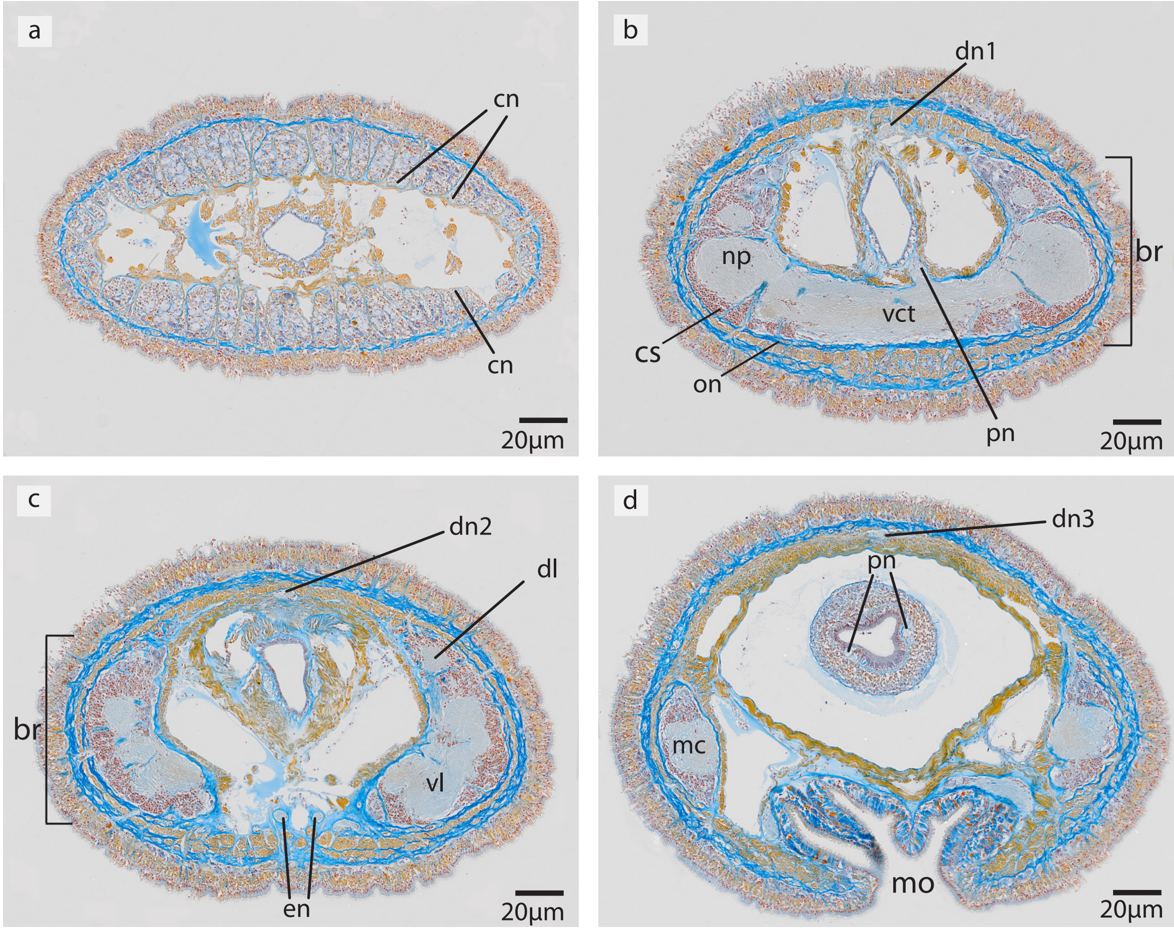


Fig. 31: *Carinoma mutabilis*, LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are circular arranged around the inner margins of the head. **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*), the whole brain is encircled by an outer neurilemma (*on*), the two proboscidial nerves (*pn*) originate from the ventral commissural tract (*vct*). *dn*: dorsal nerve. **c**: A dorsal nerve (*dn*) arises from the dorsal commissural tract, the esophageal nerves (*en*) originate from the ventral commissural tract, the brain is in its posterior part divided into a dorsal (*dl*) and ventral (*vl*) lobe. **d**: The ventral lobes are confluent with the lateral medullary cords (*mc*), two proboscidial nerves are present (*pn*). *mo:* mouth opening. *note* the different positions of the dorsal nerve (dn1- dn3).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *C. mutabilis* are circular arranged around the head lacuna and embedded in the head musculature (figs. 30b; 31a). The nerves are separated from each other by an inner neurilemma. A dorsal nerve originates from the dorsal commissural tract (fig. 31c). Anteriorly the dorsal nerve is located in the musculature, to the posterior it is located beneath the basal lamina of the epidermis (figs. 31b- c). A ventral nerve is not present. The medullary cords and the dorsal nerve are occasionally connected by very fine dorsal and ventral commissural tracts. The esophageal nerves arise from the ventral part of the ventral commissural tract and are connected just before the mouth opening by a small commissural tract (fig. 31c). Then they run to the posterior along the lateral part of the mouth opening. On the level with the esophagus they are trifurcated.

The paired proboscidial nerves originate in the ventral commissural tract and run on both sides of the proboscis connected by a proboscidial nerve plexus.

Sensory structures

No sensory structures such as eyes or cerebral organs are found in *C. mutabilis*. The cells found on the dorsal tip of the brain (fig. 32a) may represent glandular cells and might have a neurosecretory function.

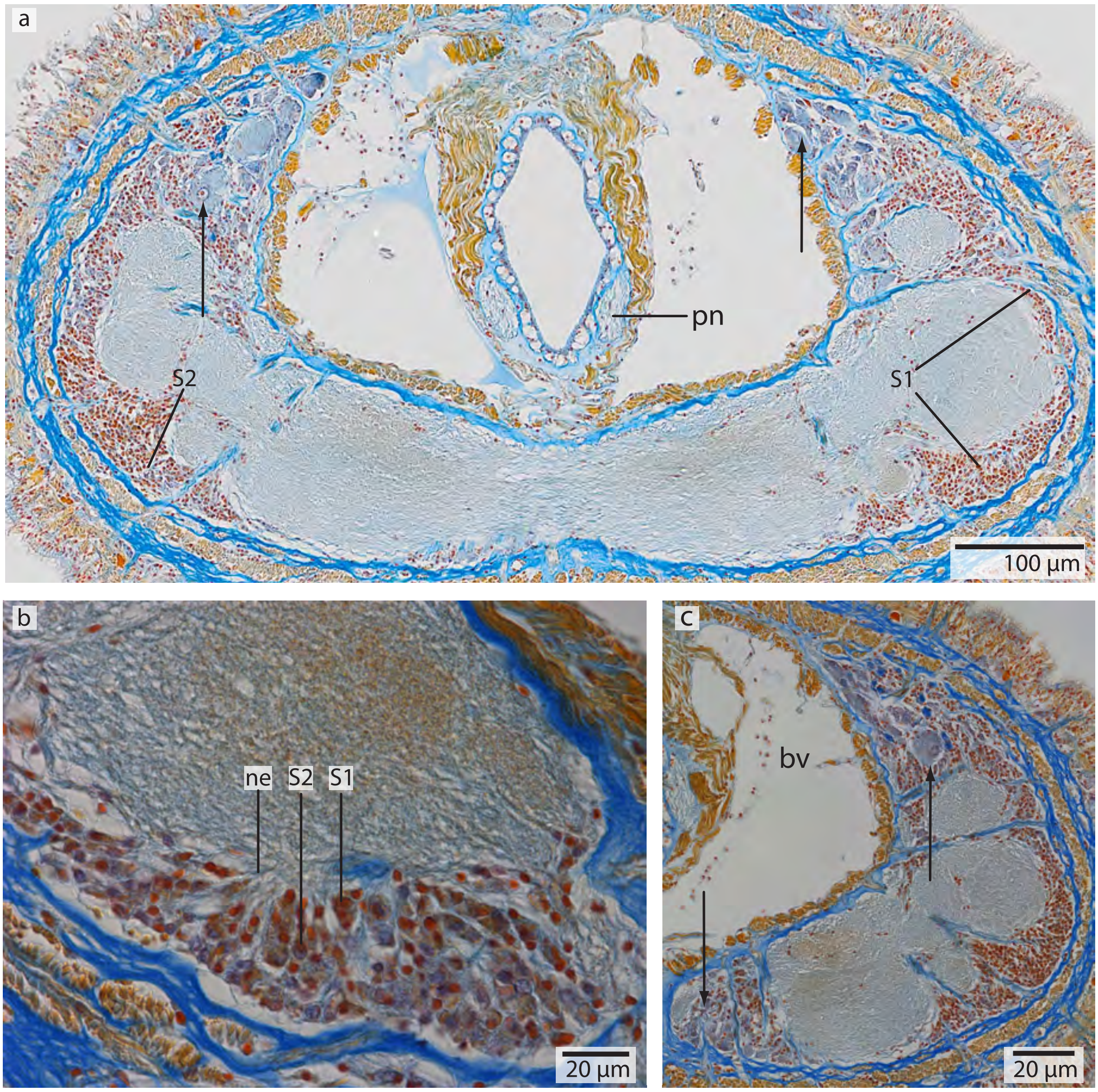


Fig. 32: *Carinoma mutabilis*, LM, cross sections, Azan. **a**: Overview of the brain region showing the location of the different cell somata types 1-2 (*S1-S2*). Another type of cells is located in the dorsal tip of the brain (*arrow*). *pn*: proboscis nerv. **b**: Higher magnifcation of neuronal cell somta type *2* (*S2*). The nuclei of *S2* dye *purple*. The neurites (*ne*) of the brain neuropil branch into the neuronal cell somata layer. **c**: in the dorsal and ventral tip of the brain, cells are found which nuclei dye *red* and the cell body is very prominent (*arrows*). Note the close association of these cell with the blood vessels (*bv*). The apperance of the cells resembles glandular cells.

3.1.8 *Lineus ruber* (Lineidae, Heteronemertea)

Lineid Heteronemerteans are characterized by the possession of prominent cephalic slits which are located on the margins of the tip of the head (Gibson 1982). The brain is situated closely anterior to the mouth opening and embedded in the head musculature. It is not externally visible (fig. 6c).

Brain and central nervous system

In its posterior part the brain is divided into a dorsal and ventral lobe. The two halves of the brain are interconnected by commissural tracts which proceed below or above the rhynchocoel respectively. The dorsal commissural tract is located on the same level as the ventral one which is more prominent (fig. 33b). The dorsal lobe is again divided into a superior and inferior strand. The inferior lobe is connected to the cerebral organ, the superior lobe ends blindly (fig. 33c). The ventral lobe of the brain gives rise to the lateral medullary cords (fig. 33d; 35d). The neuronal cell somata are separated by an inner neurilemma from the neuropil of the medullary cords. They surround the neuropil in a u-shaped manner and cap it to the exterior (fig. 33d).

The brain is composed of a central neuropil (fig. 35a) which is surrounded by neuronal cell somata. There are at least 3 types of neuronal cell somata discernable (figs. 34a- c). All nuclei of the neuronal cell somata dye dark purple. The cell bodies of neuronal cell somata type 1 are small and circular. These cells appear in clusters, primarily on the dorsal lobe of the brain (figs. 34a, b). The cell bodies of type 2 neuronal cell somata are more slender than the latter. This type primarily appears on the ventral lobe of the brain shortly behind the ventral commissural tract (figs. 34a, c). The cell bodies of type 3 neuronal cell somata are more prominent than the latter two and do not appear in clusters (figs. 34a, b). The neuronal cell somata are separated by an inner neurilemma from the neuropil of the brain. The whole brain is surrounded by an outer neurilemma (fig. 34a).

Minor nerves and peripheral nervous system

The numerous cephalic nerves originate from the lateral aspects of the brain (fig. 33a). They surround the rhynchocoel and are not covered by cell somata. A dorsal nerve originates from the middle part of the dorsal commissural tract (fig. 33d). A ventral nerve is not present. The esophageal nerves originate from a ventral commissural tract that connects paired nerves which originate in the ventral lobes of the brain (fig. 34d). The paired proboscis nerves originate in the ventral commissural tract (fig. 34a).

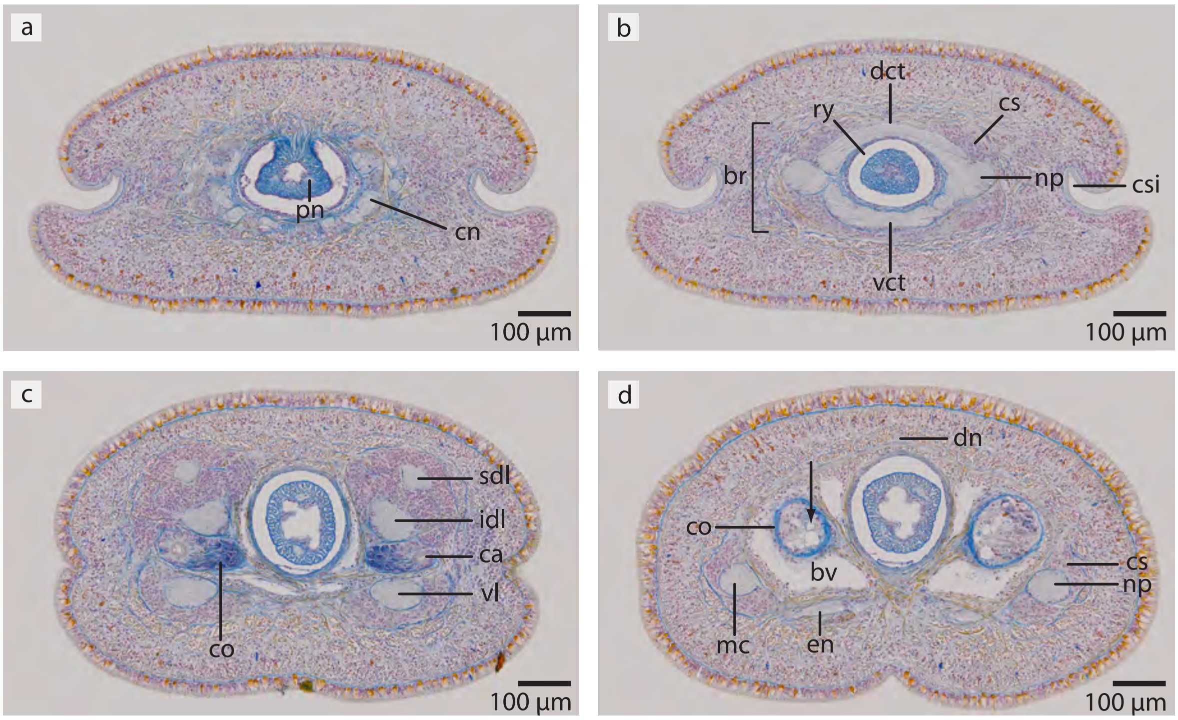


Fig. 33: *Lineus ruber*, LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are arranged in groups around the rhynchocoel. Two proboscidial nerves (*pn*) are present. **b**: The brain (*br*) is composed of a central neuropil (*np*) and surrounding neuronal cell somata (*cs*). A ventral (*vct*) and dorsal commissural tract (*dct*) connects the two halves of the brain above or below the rhynchocoel (*ry*), respectively. The cephalic slits (*csi*) connect the cerebral organ to the environment. **c**: The canal (*ca*) of the cerebral organ (*co*) ends in a layer of neuronal cell somata and glandular cells. The cerebral organ is attached to the inferior dorsal lobe (*idl*) of the brain. The superior dorsal lobe (*sdl*) rests on the cells of the cerebral organ. The ventral lobes (*vl*) of the brain give rise to the lateral medullary cords. **d**: In its posterior part the cerebral organ (*co*) is in close contact to the blood vessels (*bv*). The cells here represent glandular cells (*arrow*). The two esophageal (*en*) nerves arise from the ventral commissural tract. The neuronal cell somata (*cs*) surround the neuropil (*np*) of the medullary cord (*mc*) in a u- shaped manner.

Several nerve plexus are present in *L. ruber* (figs. 30b, c). The neurites of the intraepidermal nerve plexus are arranged in a regular, ladder-like manner. The neurites of the subepidermal, commissural plexus and the subrastomatogastric nerve plexus are horizontally arranged. The neurites of the intrastomatogastric nerve plexus are arranged in an irregular, net-like manner. The subrastomatogastric and the intrastomatogastric as well as the subepidermal nerve plexus are interconnected (fig. 35c). There is a spherical concentration of neurites in the subepidermal plexus (fig. 35c).

Sensory structures

Frontal organ

The frontal organ of *L. ruber* consists of two laterally and one dorsally located pit which are located on the tip of the animal´s head. The pits taper down to canals which end blindly in a layer of neuronal cell somata.

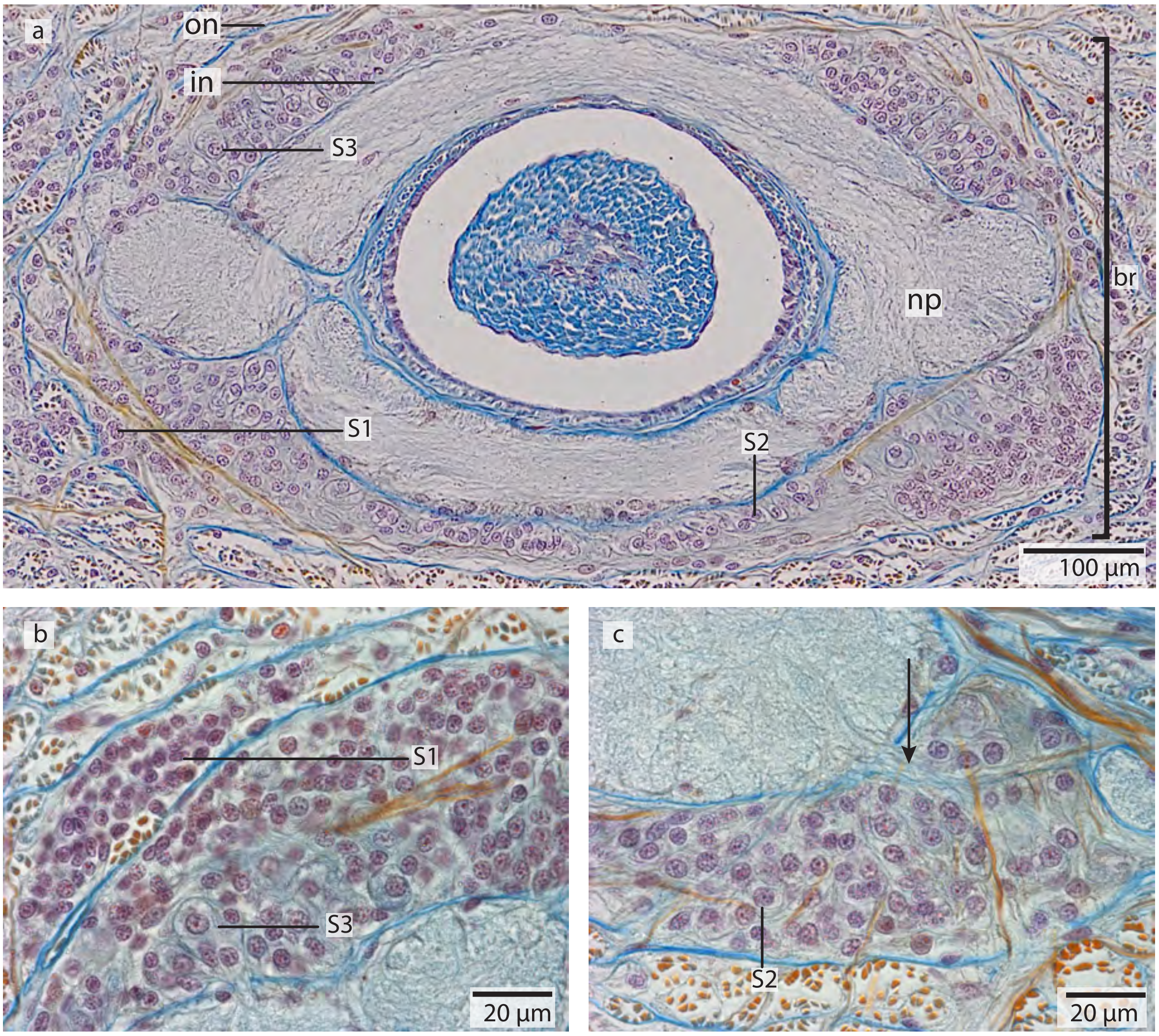


Fig. 34: *Lineus ruber*, LM, cross sections Azan. **a**: Three different types of neuronal cell somata (*S1* - *S3*) are discernable. The neuronal cell somata are separated from the neuropil by an inner neurilemma (*in*). The whole brain (*br*) is enclosed by an outer neurilemma. **b**: Nuclei of neuronal cell somata type 1 (*S1*) stain *bright purple*. The cell body is not enlarged. The cell body of neuronal cell somata type 3 (*S3*) is very prominent, the nuclei also stain *bright purple*. **c**: The cell body of neuronal cell somata type 2 (*S2*) is also enlarged, but not that prominent as in type 3 (*S3*). The nuclei dye *bright purple*. The neurites of the brain neuropil branch into the neuronal cell somata layer (*arrow*).

Cerebral organ

The cerebral organs comprise three parts: An epidermal canal which opens to the environment, a convoluted duct which runs below the basal lamina of the epidermis and in a cluster of neuronal cell somata and glandular cells in which the duct terminates (figs. 33c, d).

The neuronal cell somata and the glandular cells are enclosed by an outer neurilemma and are in close contact to the blood vessels. The inner part of the epithelium of the epidermal canal is lined with sensory cells; the outer part is densely ciliated (figs. 35a; 36a- d). The epidermal canals open into the cephalic slits which are located on the lateral margins of the head. The epidermal canal tapers down to a ciliated duct, which is located below the basal lamina of the epidermis.

The duct runs to the posterior of the animal and is convoluted (fig. 36d). The cells lining the canal, as well as those of the duct are connected by small neurites showing immunoreactivity against α-tubulin to the brain (figs. 36a, b, d). The duct terminates in a layer of neuronal cell somata and glandular cells (figs. 33c, d). These cells are innervated by neurites of the inferior dorsal lobe of the brain. The superior lobe rests on the cells of the cerebral organ (fig. 33c).

The neuronal cell somata of the cerebral organ are of type 1 described for the brain (fig. 33c) and show no immunoreactivity against FMRF (figs. 36a- d).

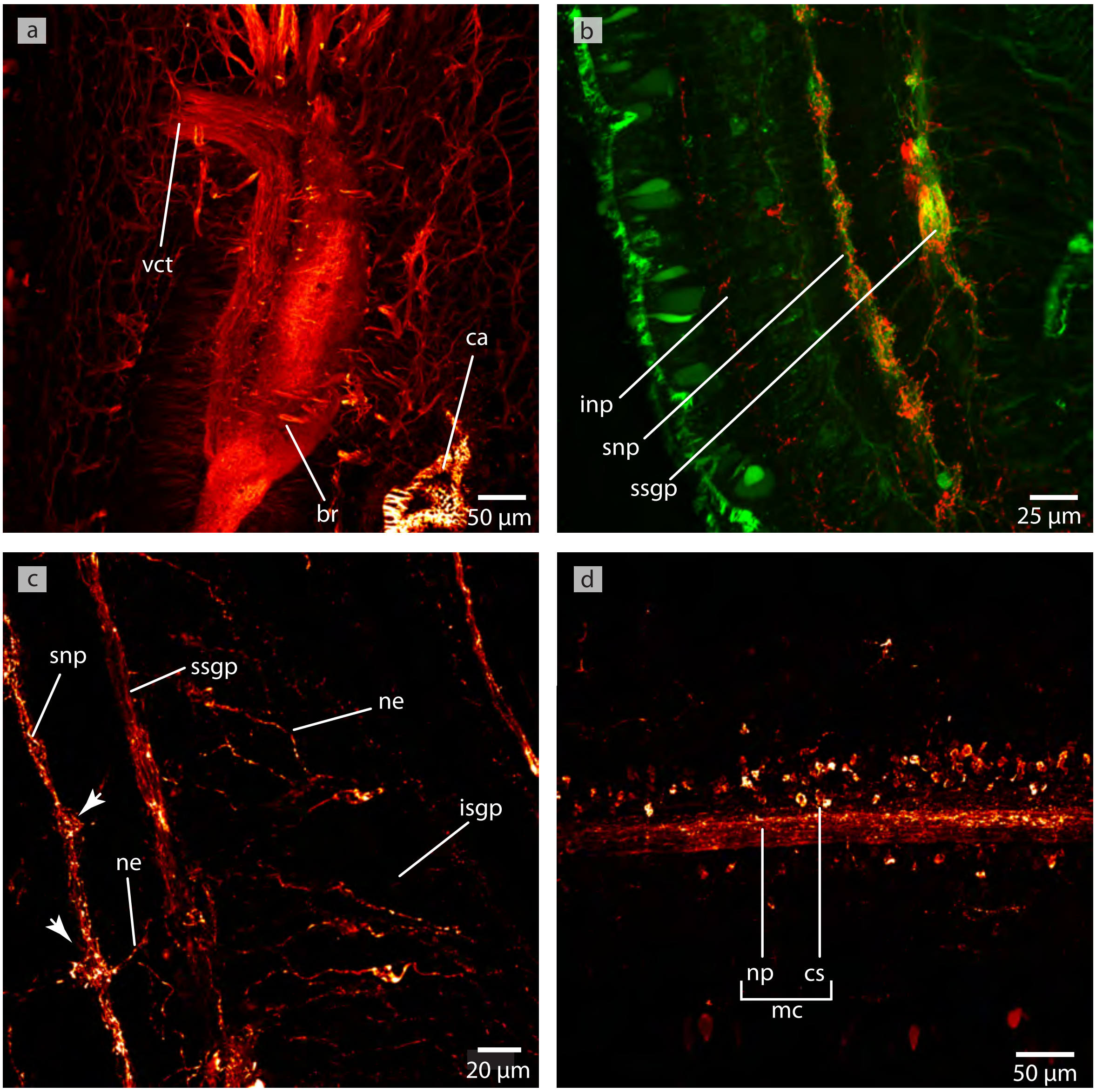


Fig. 35: *Lineus ruber*, CLSM. **a**: anti α-tubulin. The neurites of the ventral commissural tract (vct) are horizontally arranged. *br*: brain, *ca*: canal of the cerebral organ. **b**: anti FMRF (*red*), anti α-tubulin. There are several nerve plexus present in *L. ruber*. The intraepidermal (*inp*), the subepidermal (*snp*) and the suprastomatogastric (*ssgp*) nerve plexus. **c**: anti FMRF. higher magnification of *fig. b*. The subepidermal, the suprastomatogastric (*ssgp*) and the intrastomatogastric plexus are connected to each other by fine branches of neurites (*ne*). Note the concentration of neurites in the subepidermal nerve plexus (*arrows*). **d**: anti FMRF. The lateral medullary cords (*mc*) are composed of an inner neuropil (*np*) and the surrounding neuronal cell somata (*cs*).

The glandular cells are present in the posterior part of the cerebral organ close to the blood vessels. These cells are merely separated from the blood vessels by an *ecm* (figs. 33c, d).

Eyes

Eyes are numerous and of pigment cup type. They are arranged on the margins of the head.

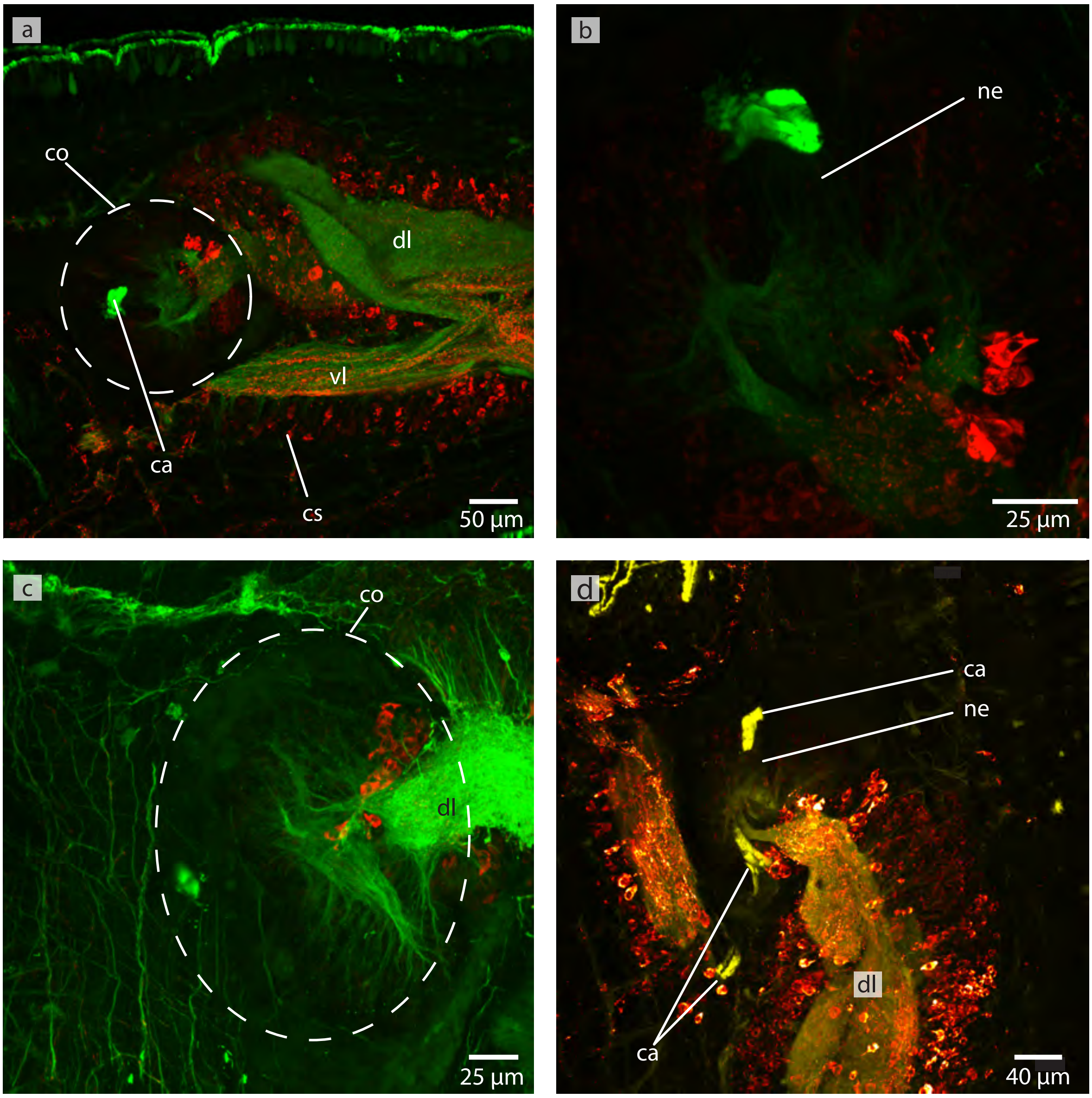


Fig. 36: *Lineus ruber*, CLSM. **a**: anti α-tubulin (*green*), anti FMRF (*red*), horizontal section. The cerbral organ (*co*, *dashed oval*) is attached to the dorsal lobe (*dl*) of the brain **b**: anti α-tubulin (*green*), anti FMRF (*red*), cross section. The cells surrounding the canal have contact to the dorsal lobe of the brain by neurites (*ne*). **c:** anti α-tubulin (*green*), anti FMRF (*red*), horizontal section. The neurites of the dorsal lobe of brain are fine branched. The cell somata of the cerebral organ (*co*, *dashed oval*) show no immunoreractivity against FMRF. **d**: anti α-tubulin (*yellow*), anti FMRF (*red*), horizontal section. The canal (*ca*) of the cerebral organ is densly ciliated and convoluted. The sensory cells surrounding the canal have contact to the brain by small neurites (*ne*). *dl:* dorsal lobe.

3.1.9 *Micrura purpurea* (Lineidae, Heteronemertea)

The brain of *Micrura purpurea* is located closely anterior to the mouth and embedded in the head musculature. It is not externally visible.

Brain and central nervous system

The posterior part of the brain is divided into a dorsal and a ventral lobe (fig. 37a). The two halves of the brain are interconnected by commissural tracts which proceed below or above the rhynchocoel respectively (fig. 37b). The dorsal commissural tract arises prior to the more prominent ventral commissural tract. The dorsal lobe is again divided into a superior and inferior strand. The inferior is connected to the cerebral organ, the superior ends blindly (fig. 37b). The ventral lobe of the brain gives rise to the lateral medullary cords. The lateral medullary cords run to the posterior of the animal between the outer longitudinal muscle layer and the inner circular muscle layer. The neuronal cell somata of the lateral medullary cord are separated by an inner neurilemma from the neuropil (fig. 37d). They resemble the type 1 neuronal cell somata described for the brain. The neuronal cell somata are u-shaped arranged around the neuropil and cap it to the exterior.

The brain of *M. purpurea* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata (figs. 37b; 38a, b). There are at least 3 types of neuronal cell somata discernable (fig. 38a). Nuclei of type 1 cell somata stain red. Nuclei of type 2 stain purple (fig. 38b). The cell body of type 3 cell somata is enlarged compared to the latter two. Nuclei stain orange (fig. 38b). The neuronal cell somata are separated by an inner neurilemma from the neuropil of the brain. The whole brain is surrounded by an outer neurilemma (fig. 38a).

Minor nerves and peripheral nervous system

The numerous cephalic nerves originate in the lateral aspect of the brain and are arranged in groups around the rhynchocoel. A dorsal nerve arises from the middle part of the dorsal commissural tract. A ventral nerve is not present. The esophageal nerves arise from the ventral commissural tract (fig. 37d). There are two proboscidial nerves present which run opposed to each other along the proboscis (fig. 37b). The nerves originate in the ventral commissural tract.

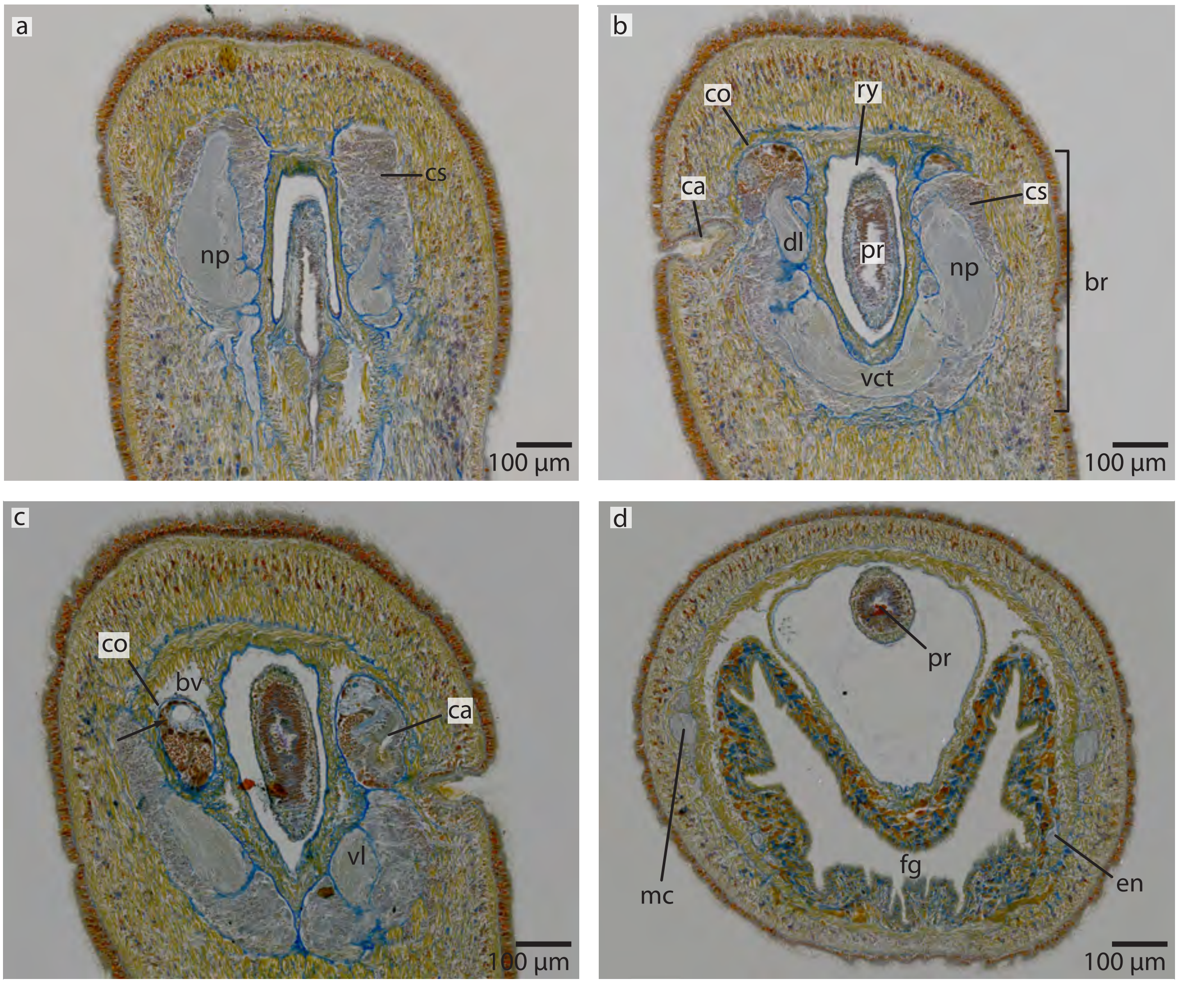


Fig. 37: *Micrura purpurea*, LM, cross sections, Azan. **a**: The brain is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*). **b**: A canal (*ca*) connects the cerebral organ (*co*) to the environment. The neuropil (*np*) of the dorsal lobe (*dl*) of the brain (*br*) is connected to the cerebral organ. The two parts of the brain are interconnected by a ventral commissural tract (*vct*) below the rhynchocoel (*ry*). **c**: The cerebral organ (*co*) is in close contact to the blood vessel (*bv*). Glandular cells (*arrow*) are merely separated by a small layer of *ecm* to the blood vessels. *vl:* ventral lobe. **d**: the ventral lobes of the brain are confluent with the lateral medullary cords (*mc*). *en:* esophageal nerve, *fg:* foregut, *pr:* proboscis.

Sensory structures

Cerebral organ

The cerebral organ is composed of a ciliated duct which ends in a layer of cell somata and glandular cells and a ciliated epidermal canal. The epithelia of the canal as well as of the duct are interiorly lined with receptive cells (figs. 37b, c) and externally with cilia. The canals open to the environment into the cephalic slits (figs. 37b, c) which are located laterally on the margins of the head. The canals taper down to ducts which are now below the basal lamina of the epidermis. The cerebral organ is attached to the inferior dorsal lobe of the brain, while the superior dorsal lobe rests on the cells of the cerebral organ. In the anterior part of the cerebral organ the cell bodies are small and the nuclei dye red and bright purple (fig. 38c).

To the posterior of the cerebral organ on the level where the ciliated duct ends, the cells become much more voluminous and represent glandular cells (fig. 37c). Here the cells are in close proximity to the blood vessels merely being separated by a small layer of extracellular matrix (figs. 37b, c).

Eyes

No pigmented eyes are present in *M. purpurea.*

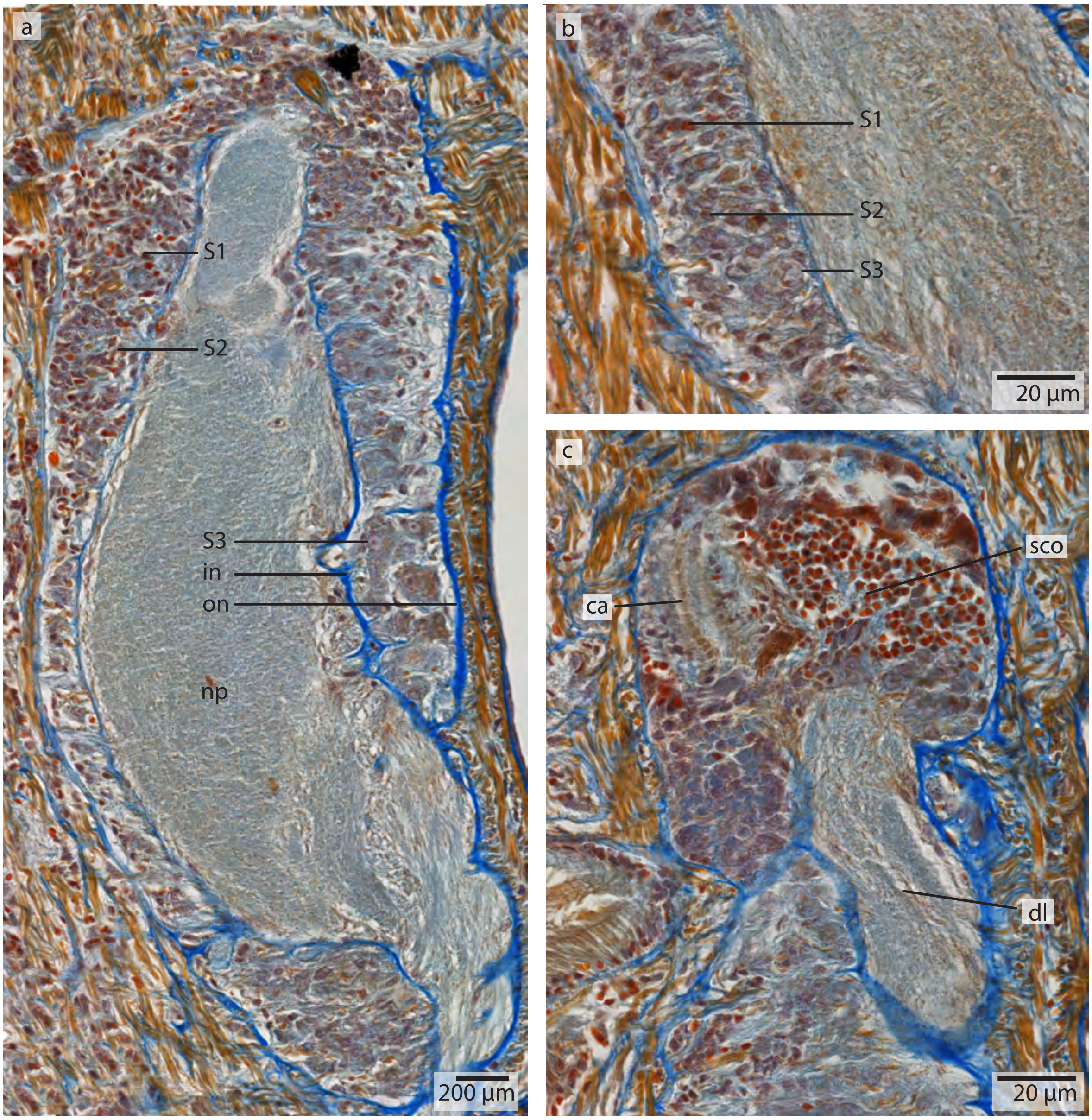


Fig. 38: *Micrura purpurea*, LM, cross sections, Azan. **a**: Three different neuronal cell somata are discernable (*S1* - *S3*). The neuronal cell somata are separated by an inner neurilemma (*in*) to the neuropil (*np*) of the brain. The whole brain is enclosed by an outer neurilemma (*on*). **b**: Nuclei of cell somata type 1 (*S1*) are circular and stain *red*. Nuclei of *S2* dye *purple*. Nuclei of type 3 (*S3*) neuronal cell somata dye *orange* and the cell body is more enlarged than in type 1 (*S1*) and 2 (*S2*). **c**: Higher magnification of the cerebral organ. The canal (*ca*) is surrounded by cell somata of the cerebral organ (*sco*), these cells resemble the type 1 described for the brain. The cerebral organ is attached to the dorsal lobe (*dl*) of the brain.

3.1.10 *Riseriellus occultus* (Lineidae, Heteronemertea)

The brain of *Riseriellus occultus* is located far anterior to the mouth opening and embedded in the head musculature. It is not externally visible (figs. 39a, b).

Brain and central nervous system

In its posterior part the brain is divided into a dorsal and ventral lobe. The two halves of the brain are interconnected by commissural tracts which proceed below or above the rhynchocoel respectively (fig. 40b). The dorsal commissural tract arises prior to the more prominent ventral commissural tract. The dorsal lobe is again divided into a superior and inferior strand (fig. 40c). The inferior is connected to the cerebral organ, the superior ends blindly. The ventral lobe of the brain gives rise to the lateral medullary cords (fig. 40d). The lateral medullary cords run to the posterior of the animal between the outer longitudinal muscle layer and the inner circular muscle layer. The neuronal cell somata of the lateral medullary cord are separated by an inner neurilemma from the neuropil. They resemble the type 1 neuronal cell somata described for the brain. The neuronal cell somata cap the neuropil to the exterior and are surrounded by an outer neurilemma. (fig. 40b).

The brain of *R. occultus* is composed of neuropil which is surrounded by a massive layer of neuronal cell somata (figs. 40b, c; 41a, b). There are at least three types of neuronal cell somata discernable (figs. 41a, b). All nuclei dye dark purple. The cell body of neuronal cell somata type 1 is small and circular. These cells appear in clusters, primarily on the dorsal lobe of the brain (fig. 41a). Cell body of type 2 neuronal cell somata is more slender the latter. This type primarily appears on the ventral lobe of the brain shortly behind the ventral commissural tract (fig. 41b). Cell bodies of type 3 neuronal cell somata appear larger than the latter two and are solitarily distributed over the brain (figs. 41a, b). The neuronal cell somata are divided by the inner neurilemma from the neuropil and the whole brain is surrounded by an outer neurilemma. The neurilemma entirely is composed of *ecm* indicated by the blue coloration in the Azan staining.

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *R. occultus* originate from the lateral parts of the brain and are arranged in groups around the rhynchocoel (figs. 39b; 40a). The nerves are enclosed by the inner neurilemma and neuronal cell somata are not present there.

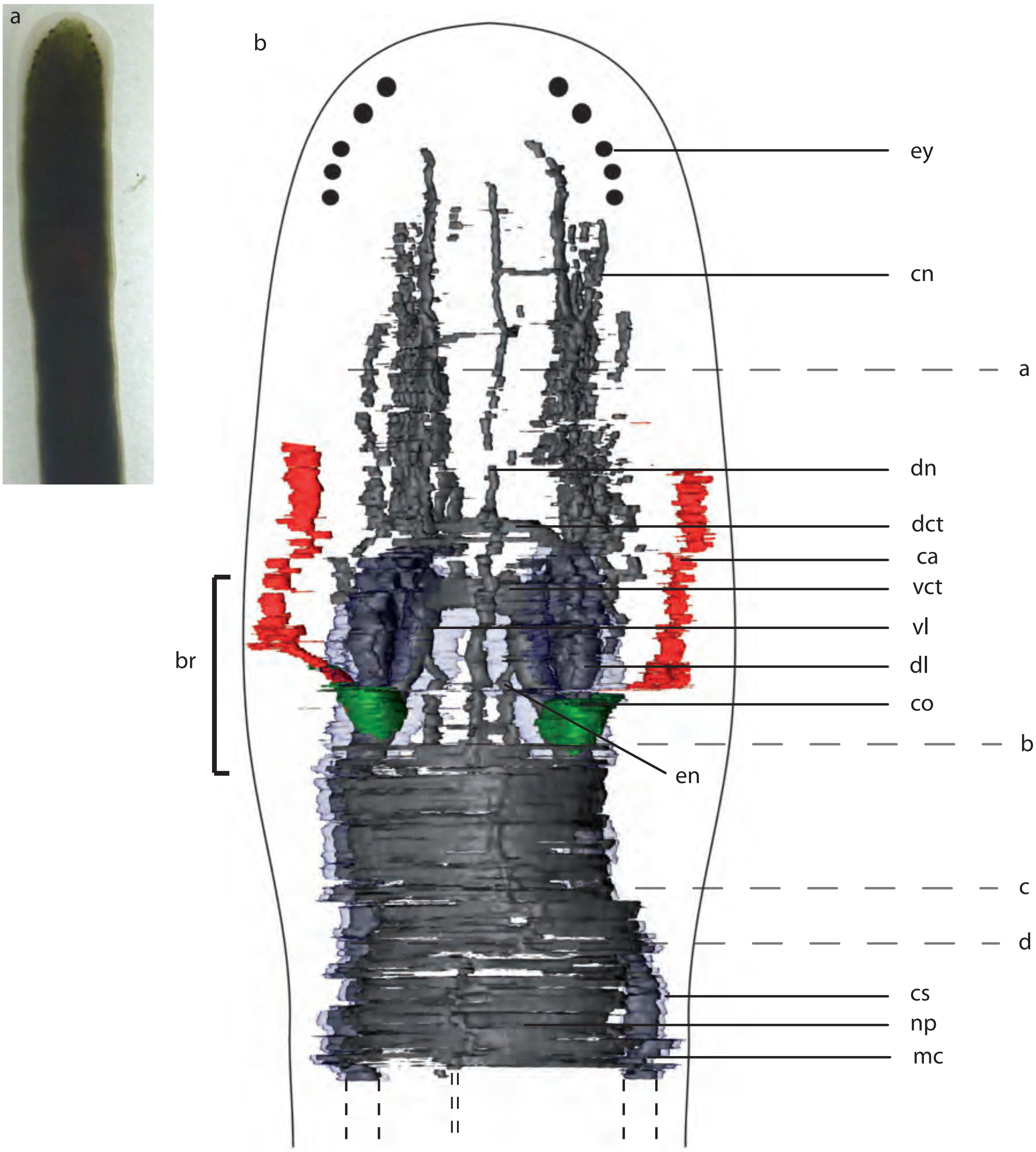


Fig. 39: *Riseriellus occultus.* **a**: Living specimen. **b**: Central nervous system, dorsal view, snapshot, 3d-reconstruction (428 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are circular arranged around the rynchocoel of the animal, a dorsal commissural tract (*dct*) arises in front of the ventral commissural tract (*vct*). The posterior part of the brain is divided into a dorsal (*dl*) and ventral lobe (*vl*). The cerebral organ (*co*) is attached to the dorsal lobe of the brain (*br*). The canals (*ca*) open to the environment. A dorsal nerve strand (*dn*) originates from the dorsal commissural tract and runs to the anterior of the dorsal commissural tract as well as to the posterior of the dorsal commissural tract. The branching esophageal nerves (*en*) originate from the ventral lobes of the brain. The lateral medullary cords (mc) originate in the ventral lobes of the brain. letters on the right (**a** - **d**) refer to the histological sections in figure 40.

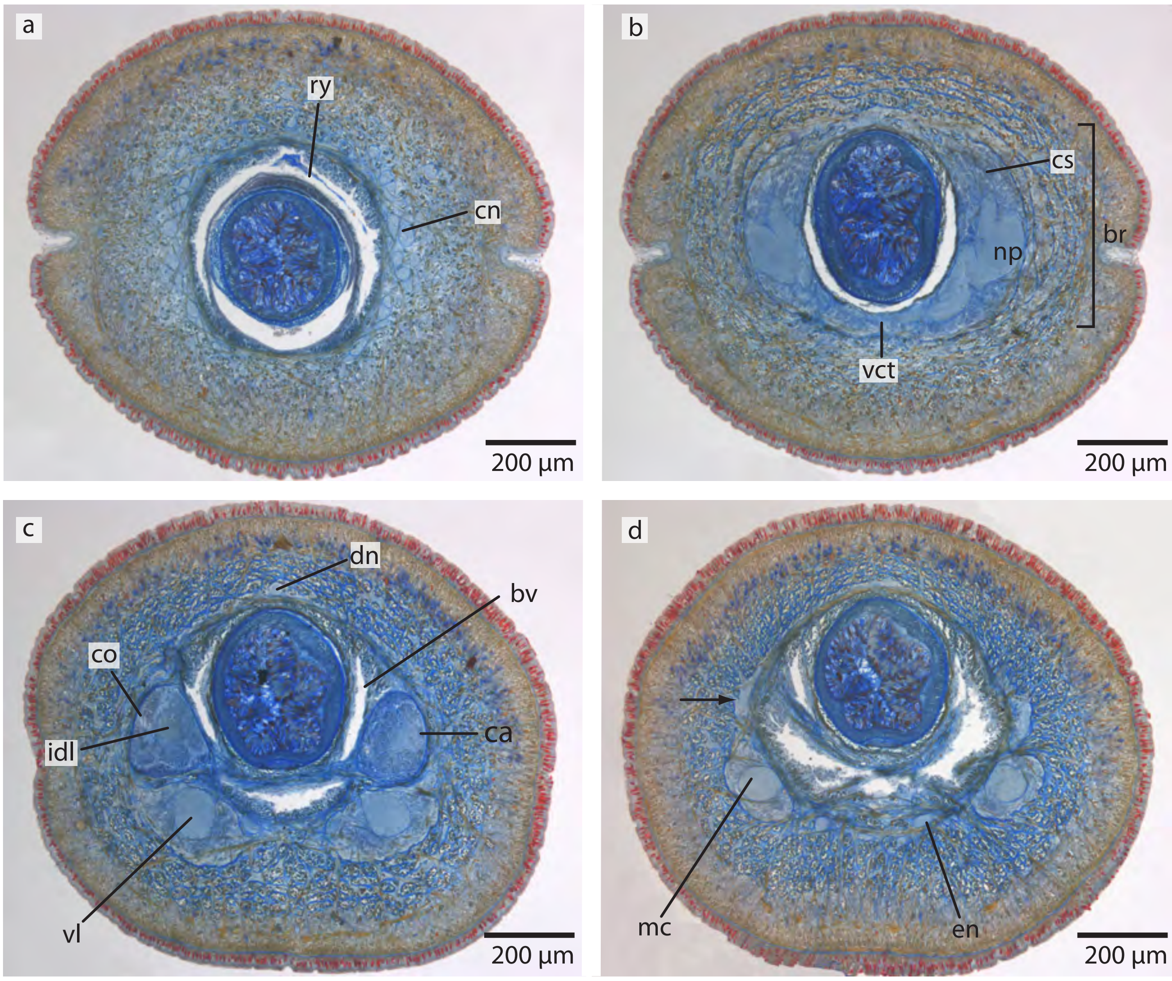


Fig. 40: *Riseriellus occultus*, LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are arranged in groups around the rhynchocoel (*ry*). **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by neuronal cell somata (*cs*). The two parts of the brain are connected by a ventral commissural tract (*vct*) **c**: The canals (*ca*) of the cerebral organ end in a layer of neuronal cell somata and glandular cells. The cerebral organ (*co*) is attached to the inferior dorsal (*idl*) lobe of the brain and in close contact to the blood vessels (*bv*). A dorsal nerve (*dn*) arises from the dorsal commissural tract. **d**: The ventral lobes of the brain are confluent with the lateral medullary cords (*mc*), a nerve plexus (*arrow*) connects the two lateral medullary cords. *en*: esophageal nerve.

A dorsal nerve arises from the middle part of the dorsal commissural tract and runs in both directions from the frontal to the caudal (figs. 39b; 40c). The medullary cords are interconnected with the dorsal nerve by a small dorsal nerve plexus, which forms a roof like structure above the rhynchocoel (figs. 39b, 40d). This plexus is present on the level of the posterior part of the cerebral organ. A ventral nerve is not present. The esophageal nerves arise from each ventral lobe of the brain (fig. 40d). They are occasionally connected by small commissural tracts. Short in front of the mouth opening the esophageal nerves fuse before they branch to run on either sides of the foregut. There are two main proboscidial nerves which are connected by small circular nerve plexus and run on both sides of the proboscis. The origin of the proboscis nerves could not be reconstructed.

Sensory structures

Frontal organ

The frontal organ of *R. occultus* consists of two laterally and one dorsally located pit which are situated on the tip of the animals´head. The pits taper down to canals which end blindly in a layer of neuronal cell somata (fig. 41c). The nuclei of these cells dye bright purple and are not found anywhere else in the central nervous system. A connection of these cells to the brain was not visible.

Cerebral organ

The cerebral organ is composed of epidermal canals and ciliated ducts which terminate in a layer of neuronal cell somata and glandular cells. Both epithelia of the canal and the duct are interiorly lined with sensory cells. The epidermal canal opens lateral into the cephalic slits (fig. 39b) and tapers to the inner into a duct which runs below the basal lamina of the epidermis. In the anterior part of the duct of the cerebral organ the cells are small and dye dark purple (fig. 40c). The duct is convoluted and to the posterior of the cerebral organ on the level where the ciliated duct ends, the cell become much more voluminous. Here the cells of the cerebral organs are in close proximity to the blood vessels merely being separated by a small layer of extracellular matrix (fig. 40c). The cerebral organ is innervated by neurites of the inferior lobe of the dorsal lobe of the brain (fig. 40c). The superior lobe of the dorsal lobe of the brain rests on the cells of the cerebral organ.

Eyes

Eyes are numerous and are arranged on the margins of the head (figs. 39a, b). The eyes are of the pigment-cup type. No direct contact of the brain and the eyes could be observed.

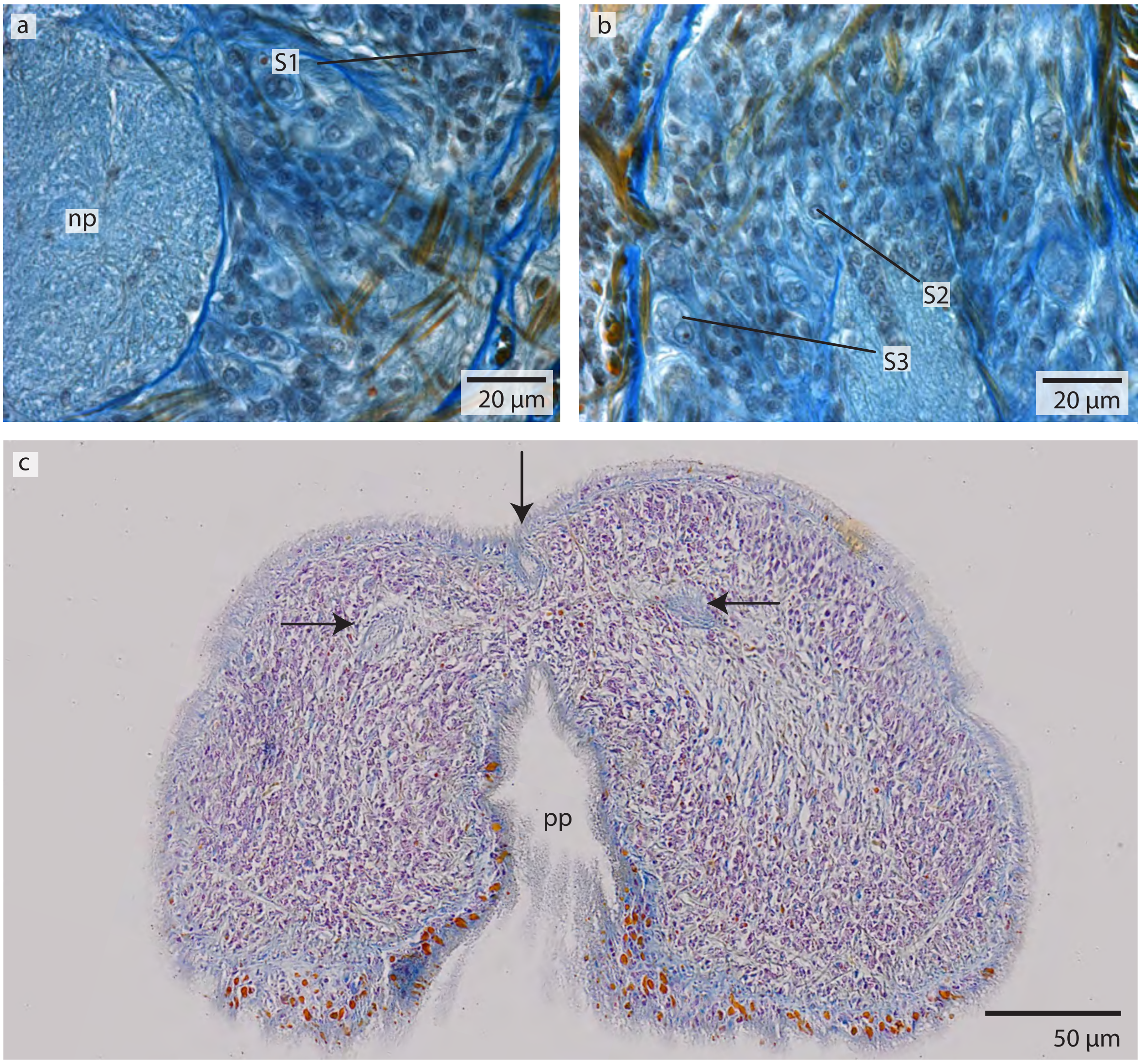


Fig. 41: *Riseriellus occultus*, LM, cross sections, Azan. **a**: The nuclei of cell somata type 1 (*S1*) stain *dark purple* and appear in cluster. **b**: The cell body of neuronal cell somata type 2 (*S2*) is enlarged compared to type 1 (*S1*), the nuclei stain *bright purple*. Cell bodies of neuronal cell somata 3 (S3) are much more prominent than in *S1* and *S2*. These cells are fewest present in the brain. **c**: The frontal organ consits of two lateral and one dorsally located pit which end blindly (*arrows*). *pp* proboscis pore.

3.1.11 *Amphiporus lactifloreus* (Amphiporidae, Hoplonemertea)

The head of *Amphiporidae* is slightly demarcated from the trunk. The brain of *Amphiporus lactifloreus* is located inside the head musculature. It is externally visible as a reddish structures (fig. 42a).

Brain and central nervous system

The brain is only in its very posterior part divided into a dorsal and a ventral lobe. The two lobes are interconnected by a dorsal and ventral commissural tract respectively. A much more prominent ventral commissural tract appears prior to the dorsal one (fig. 43c). The dorsal part ends in a layer of cell somata close to the blood vessels. The ventral lobes are confluent with the lateral medullary cords (fig. 43d). The medullary cords run embedded in the longitudinal muscle layer and are in their anterior part, shortly behind the brain, in close contact to the nephridial system (fig. 43c). The neuronal cell somata are localized dorsally and ventrally to the neuropil. They resemble the neuronal cell somata type 1 described for the brain.

The brain of *A. lactifloreus* is composed of a central neuropil which is surrounded by neuronal cell somata (figs. 42b; 43c; 44a; 45b). There are at least three types neuronal cell somata discernable (figs. 44a- d). All nuclei dye purple. Type 1 neuronal cell somata is found on the dorsal lobe of the brain. These cells are circular and appear in cluster (figs. 44a, b). Type 2 neuronal cell somata are found on the ventral lobe of the brain. These cells show an enlargement of the cytoplasm and thus appear larger (figs. 44a, c). Cell bodies of type 3 neuronal cell somata are the most prominent (figs. 44c). The neuronal cell somata are not separated from the neuropil of the brain by an inner neurilemma but the whole brain is surrounded by an outer neurilemma (figs. 43c). There is no clear division of the neuronal cell somata to the neuropil, the neurites also branch into the cell somata. The neuropil of the brain shows a different arrangement of the neurites of the cell somata. In some parts especially in the commissural tracts, the neurites are horizontally arranged (fig. 44a). In the dorsal lobe of the brain the neurites form cluster of circular, spherical structures (fig. 44a). The neurites connecting the ventral with the dorsal lobe of the brain are vertically arranged (fig. 44a).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *A. lactifloreus* originate from the lateral parts of the brain (figs. 42b, 43a, b). The nerves are embedded in the longitudinal muscle layer and are surrounded by a neurilemma.

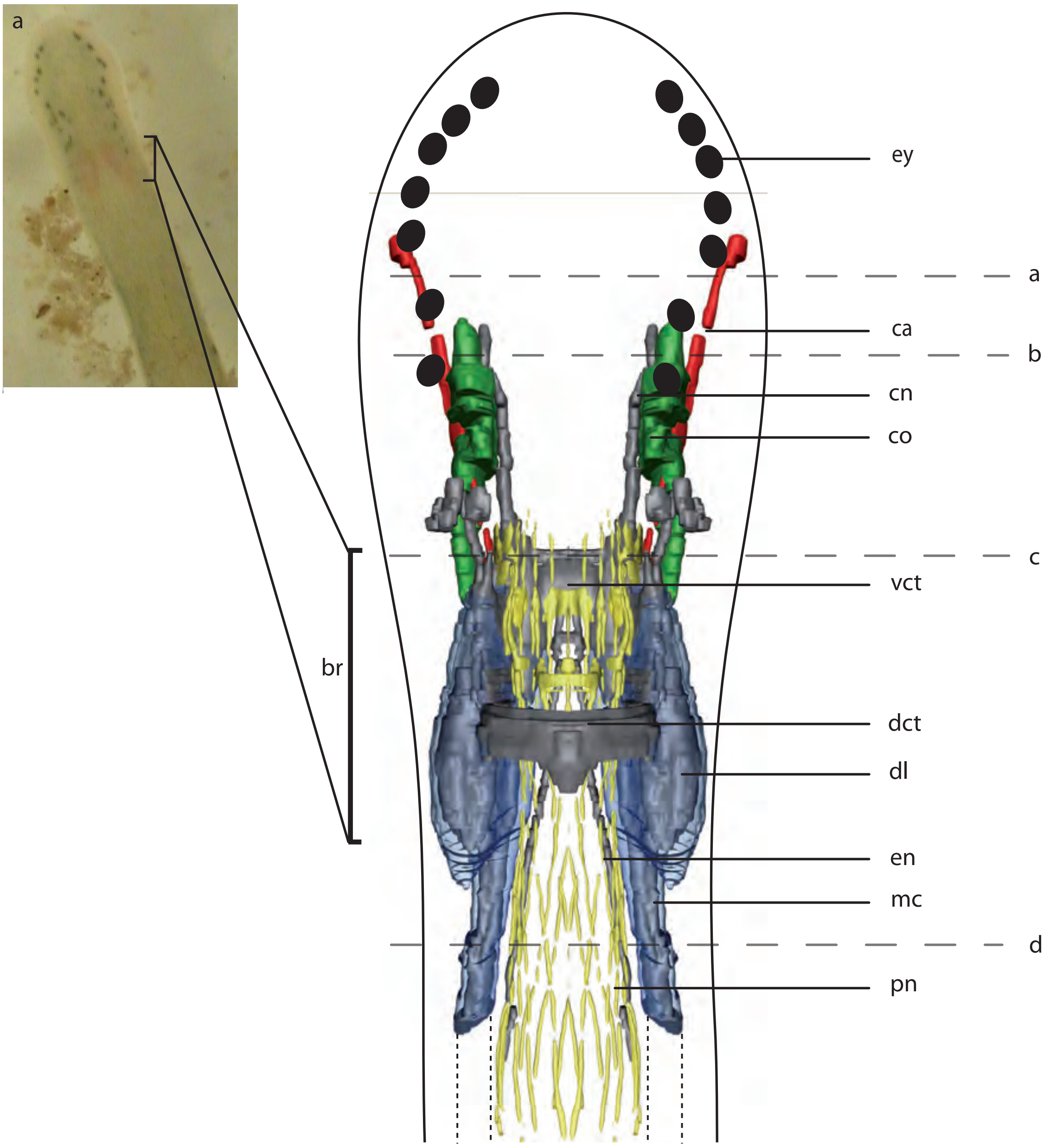


Fig. 42: *Amphiporus lactifloreus.* **a**: Living specimen, the brain (*br*) is shining through the tissue of the body wall. **b**: Central nervous system, dorsal view, snapshot 3d-reconstruction (140 slices). The nervous system is composed of neuropil (*gray*) which may be surrounded by cell somata (*blue*). Cephalic nerves (*cn*) are present. The proboscidial nerves (*pn*, *yellow*) originate from the ventral lobe of the brain. The posterior part of the brain is divided into a dorsal (*dl*) and ventral lobe. Both lobes are interconnected by a dorsal (*dct*) and ventral (*vct*) commissural tract. The canals (*ca*) of the cerebral organ (*co*) open ventro-laterally to the environment. The esophageal nerves (*en*) originate from the ventral commissural tract (*vct*). The lateral medullary cords (*mc*) originate in the ventral lobes of the brain. Letters on the right (**a** - **d**) refer to the histological sections in figure 43.

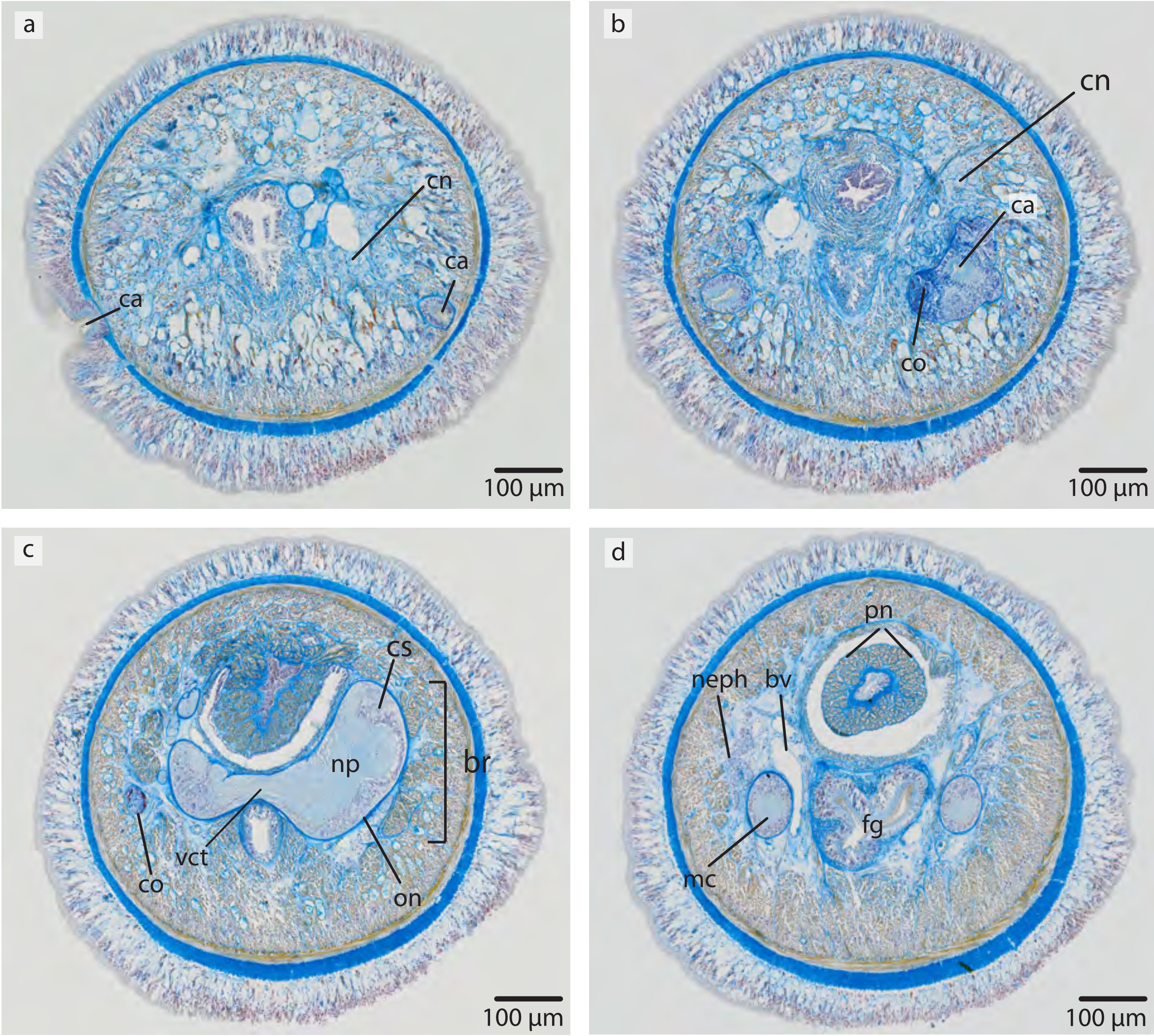


Fig. 43: *Amphiporus lactifloreus,* LM, cross sections, Azan. **a**: The canals (*ca*) of the cerebral organ open to the environment ventro- lateral. The cephalic nerves (*cn*) surround the rhynchocoel. **b**: The canal (*ca*) of the cerebral organ (*co*) ends in a layer of cell somata. The cerebral organ (*co*) is in close contact to the cephalic nerves (*np*) of the brain. **c**: The brain is composed of a central neuropil (*np*) and the surrounding cell somata (*cs*). A ventral commissural tract (*vct*) connects the two halves of the brain. The whole brain is enclosed by an outer neurilemma (*on*). **d**: The lateral medullary cords (*mc*) are in close association with the nephridia (*neph*) and the blood vessels (*bv*). 14 proboscidial nerves (*pn*) are present.

The esophageal nerves originate from the lateral parts of the ventral parts of the brain (fig. 42b). A dorsal or ventral nerve are not present. There are 14 main proboscis nerves which originate from the dorsal part of the anterior brain and are connected by a proboscidial plexus (figs. 44b; 45c).

Several nerve plexus are present in *A. lactifloreus* (fig. 45d). The neurites of the intraepidermal nerve plexus are arranged in a ladder-like way (fig. 46a), while the neurites of the intrastomatogastric nerve plexus are arranged in an irregular net-like fashion (fig. 46b). The neurites of the commissural as well as of the subrastomatogastric nerve plexus are horizontally arranged.

Sensory structures

Cerebral organ

The cerebral sense organs consist of a short ciliated epidermal canal and a duct which opens to the environment to the ventro-lateral and is lined with a layer of receptive cells (figs. 43a- c; 45a; 46c, d).

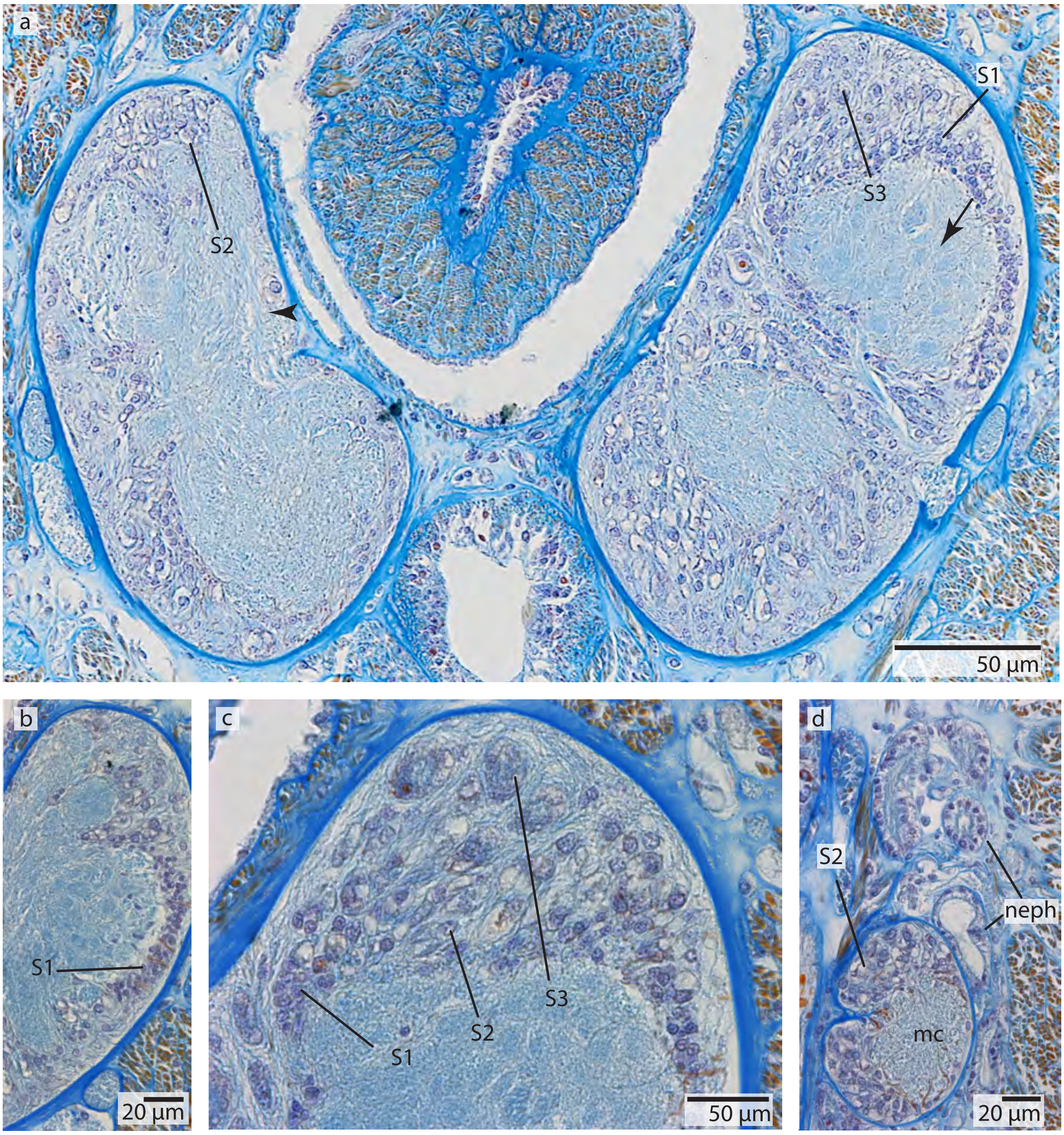


Fig. 44: *Amphiporus lactifloreus*, LM, cross sections, Azan. **a**: Overview of the brain region showing the location of the different neuronal cell somata types (*S1 - S3*). The neurites of the brain form circular structures (*arrow*) in the dorsal lobe of the brain. The neurites connecting the dorsal with the ventral lobe of the brain are vertically arranged (*arrowhead*). **b**: Higher magnifcation showing cell somata type 1 (*S1*). The nuclei of *S1* dye *purple*; the cells appear in cluster, the cytoplasm is not enlarged. **c**: Higher magnifcation of *S3* showing nuclei stained *purple*, too, and are circular but the cytoplasma is more prominent than in *S1 - S2*. **d**: The cell somata of the medullary cords (*mc*) are of the *S2* type. The medullary cords (*mc*) are in close contact to the nephridia (*neph*).

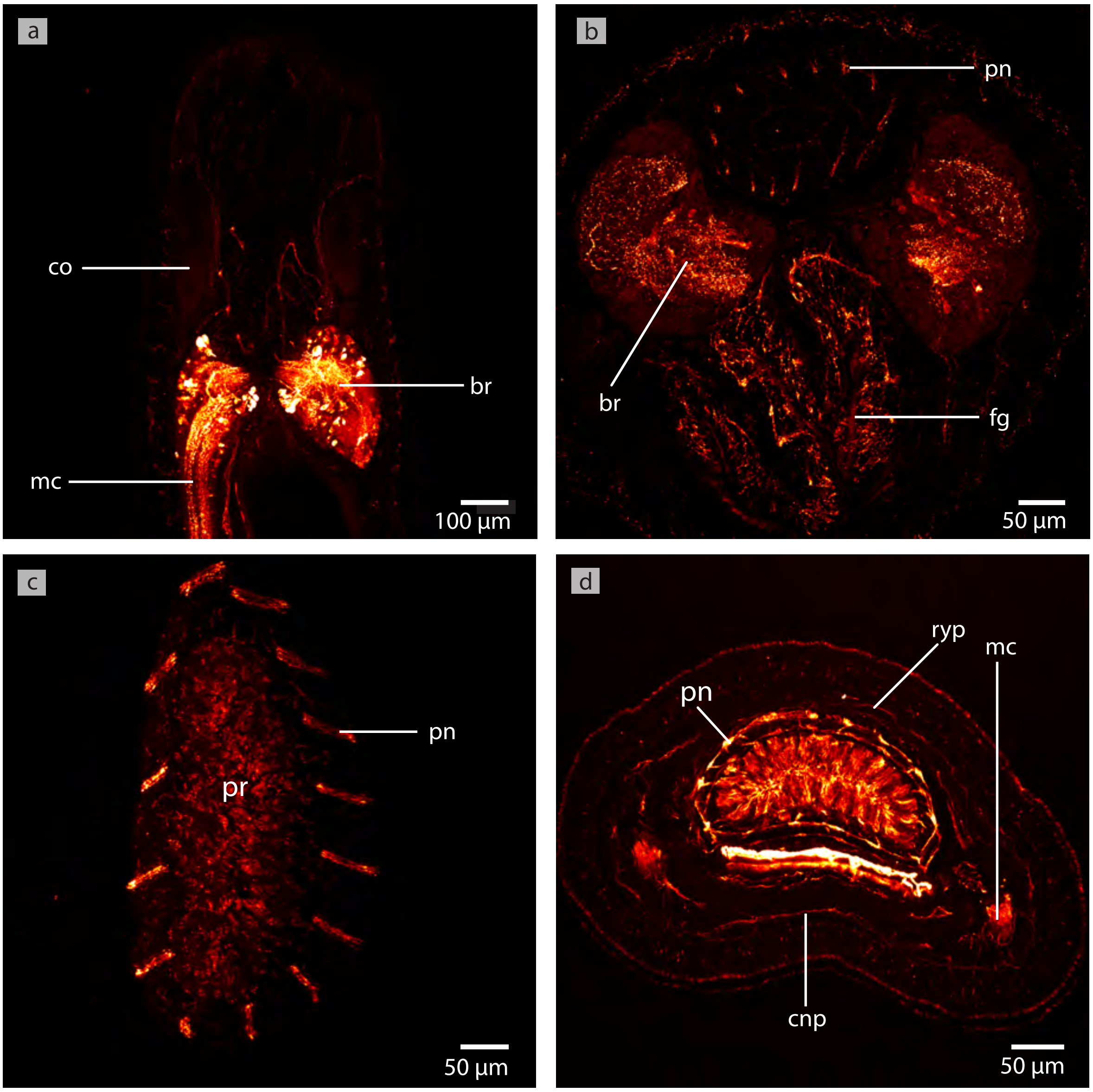


Fig. 45: *Amphiporus lactifloreus*, CLSM. **a**: Anti serotonin, horizontal section. The cerebral organ (*co*) is situated anterior to the brain (*br*). The neurites connecting the cerebral organ to the brain show no immunoreactivity against serotonin. *mc*: medullary cord. **b**: Anti serotonin, cross section. Only few neurites and cell somata are stained against serotonin. *fg*: foregut, *pn:* proboscis nerve **c**: Anti FMRF, cross section. 14 proboscidial nerves (*pn*) are present. *pr:* proboscis. **d**: Anti α-tubulin, cross section. The lateral medullary cords (*mc*) are interconnected by the commissural nerve plexus (*cnp*). The rhynchocoelan nerve plexus (*ryp*) surrounds the rhynchocoel. *mc*: medullary cord. *pn*: proboscis nerve

The duct runs to the inner part of the animal and ends blindly in a cell layer of voluminous cells. These are probably of neurosecretory function, since they are separated from the blood vessels by a thin layer of *ecm* (figs. 43b, c). The neuronal cell somata surrounding the duct are of the 1 type described for the brain (fig. 43b). The sensory cells lining the canal have contact to the brain by small neurites showing immunoreactivity against Tubulin (fig. 46c). The neurites show no immunoreactivity against FMRF or serotonin (fig. 46d).

Eyes

Eyes are numerous and of pigment cup type. They are arranged around the margins of the head (fig. 42a).

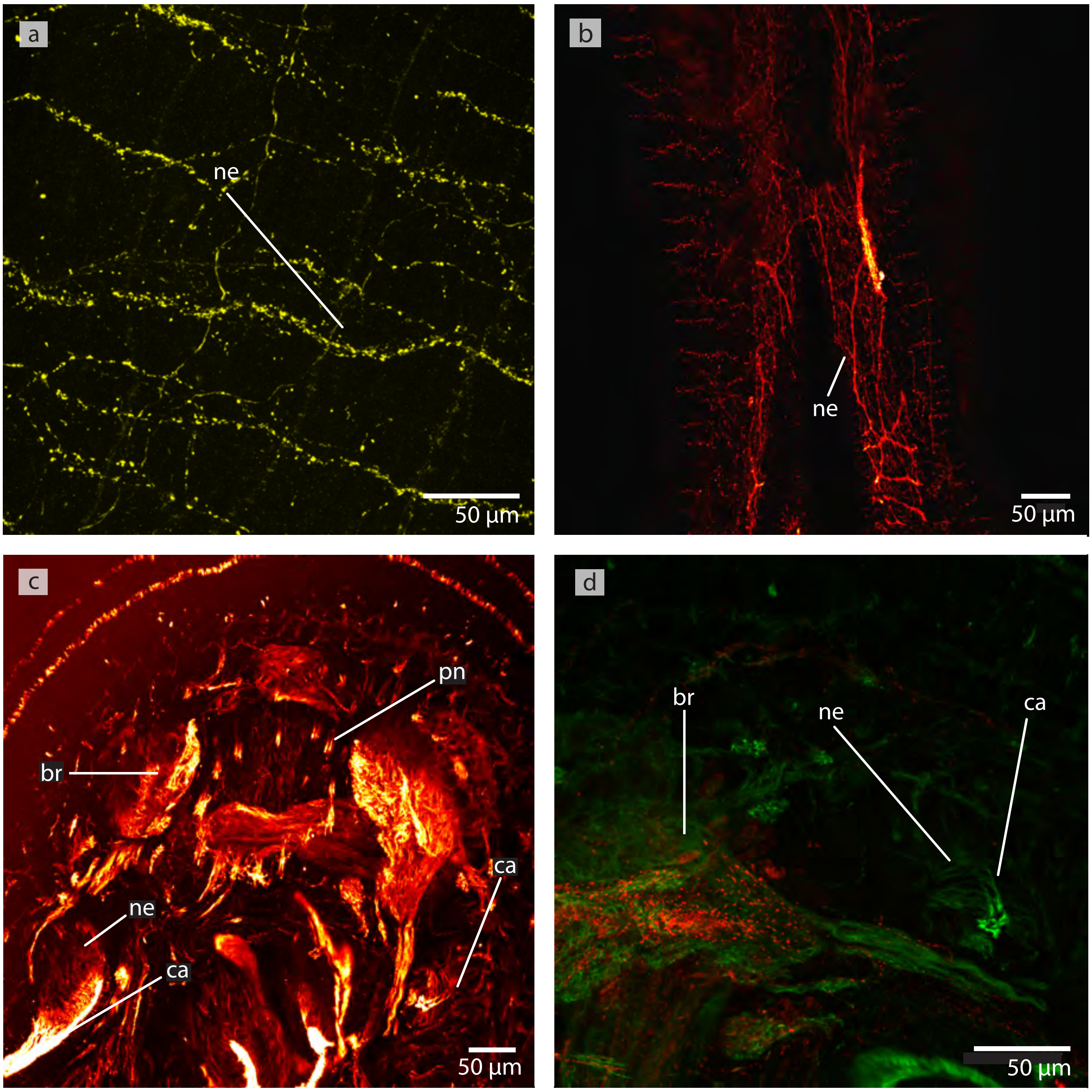


Fig. 46: *Amphiporus lactifloreus*, CLSM. **a**: Horizontal section, anti FMRF. The neurites (*ne*) of the intraepidermal nerve plexus are arranged in a regular ladder-like way. **b**: Horizontal section, anti FMRF. The neurites (*ne*) of the intrastomatogastric nerve plexus are arranged in a diffuse net-line way. **c**: Cross section, anti α-tubulin. The sensory cells lining the canal (*ca*) of the cerebral organ are connected to the brain (*br*) by branches of neurites (*ne*). **c**: Sagittal section, anti FMRF (*red*), anti α-tubulin (*green*). *pn*: proboscis nerve. **d**: The neurites (*ne*) connecting the sensory cells of the canal (*ca*) to the brain (*br*) show no immunoreactivity against FMRF.

3.1.12 *Amphiporus imparispinosus* (Amphiporidae, Hoplonemertea)

The brain of *Amphiporus imparispinosus* is located inside the head musculature.

Brain and central nervous system

The brain (fig. 47b) is divided posterior into a dorsal part and a ventral lobe. The two lobes are interconnected by a dorsal and ventral commissural tract respectively. The dorsal commissural tract is located prior to the ventral one which is more prominent. The dorsal lobes end blindly in a layer of neuronal cell somata of type 1 and 2 close to the blood vessels (fig. 47c). The lateral medullary cords originate from the ventral lobes of the brain. They run to the posterior of the animal embedded in the longitudinal muscles layer. The neuronal cell somata are not separated by a neurilemma from the neuropil. They cap the neuropil dorsally and ventrally. The neuronal cell somata are of type 2 described for the brain (fig. 48e). The medullary cords are in close proximity to the nephridial system (figs. 47d; 48e).

The brain of *A. imparispinosus* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata (fig. 47c). There are at least 3 types of neuronal cell somata present (fig. 48a). The nucleus of type 1 neuronal cell somata dyes red (figs. 48a, b). The nucleus type 2 neuronal cell somata dye purple (figs. 48a, b). This types of cell are most numerous in the dorsal part of the brain. The nucleus type 3 neuronal cell somata dyes purple and has an enlarged cell body and thus appears larger than the latter two (figs. 48a, c). A 4 type of presumably neuronal cell somata is found of the tip of the dorsal lobe of the brain (figs. 48d). The cell bodies of these cells are very large and the cytoplasm dyes purple. These cells are found in close proximity to the eyes (fig. 48d). The neuronal cell somata are not separated from the neuropil by an inner neurilemma. An outer neurilemma is clearly discernable (fig. 47c). The neurites of the brain neuropil show a different arrangement. In the commissural tracts the neurites are horizontally arranged (fig. 48a). In the dorsal part of the brain the neurites form circular structures (fig. 48d). The neurites connecting the dorsal with the ventral lobe of the brain are vertically arranged (fig. 48d).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *Amphiporus imparispinosus* originate from the lateral parts of the brain (fig. 47a). They run inside the body musculature and are enclosed by a neurilemma. There is no dorsal or ventral nerve present. The esophageal nerves arise from the ventral commissural tract and run along the foregut. The proboscis nerve consists of 16 main nerves which are connected by a proboscidial plexus.

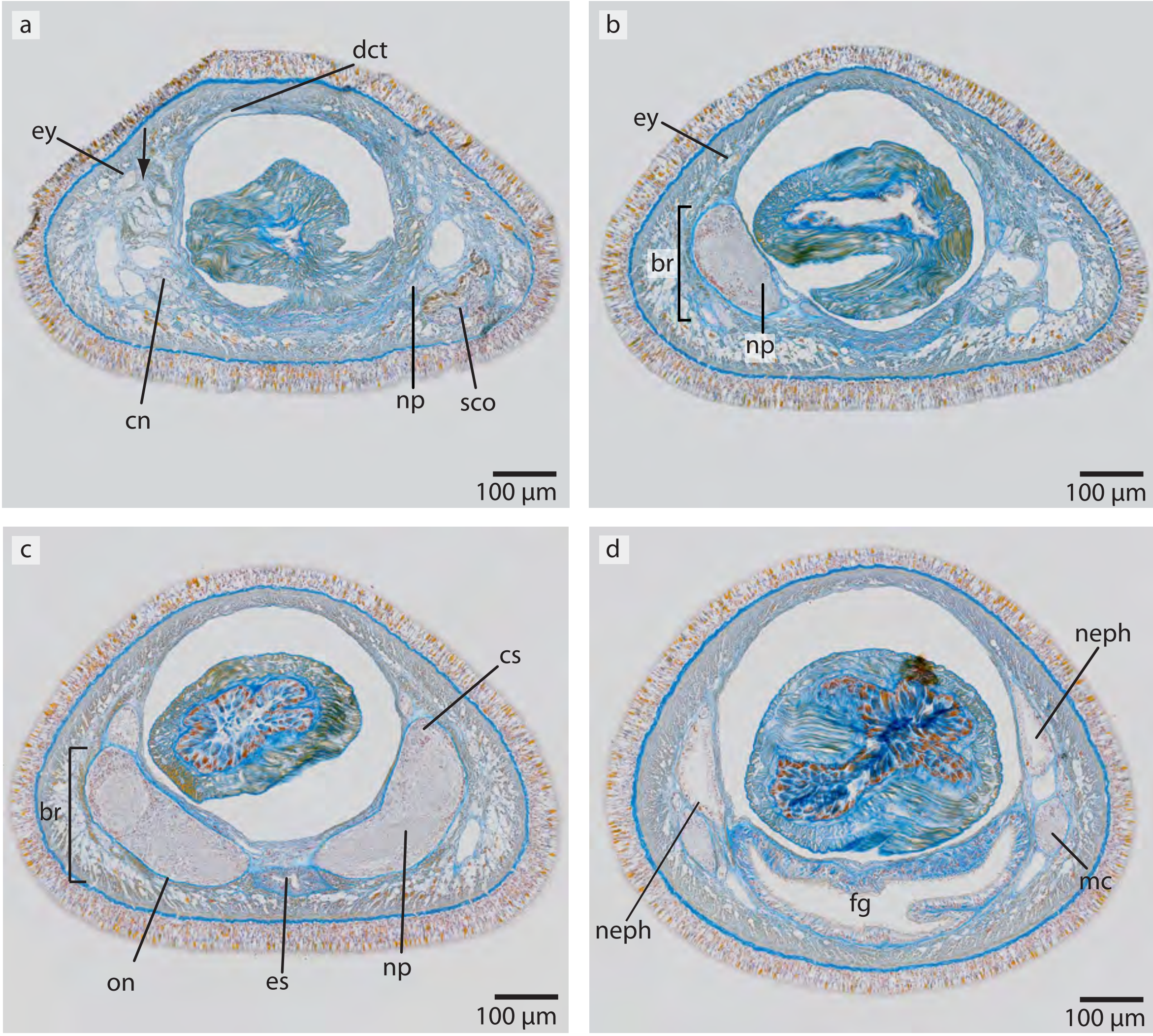


Fig. 47: *Amphiporus imparispinosus*, LM, cross sections, Azan. **a**: The cephalic nerves (*cn*) are arranged in groups around the rhynchocoel. The cell somata of the cerebral organ (*sco*) have contact to the neuropil (*np*) of the brain. The neurites of the eyes (*ey*) have contact to the neuropil of the brain (*arrow*). *dct*: dorsal commissural tract. **b**: The posterior eyes (*ey*) are in close association to neuropil (*np*) of the brain (*br*). **c**: The brain is composed of a central neuropil (*np*) which is surrounded by neuronal cell somata (*cs*) and is enclosed by an outer neurilemma (*on*). *es:* esophagus. **d**: The lateral medullary cords (*mc*) are in close association with the nephridia (*neph*). *fg:* foregut.

Sensory structures

Cerebral organ

The cerebral sense organ of *A. imparispinosus* is located anterior to the brain. The canals open ventrally to the environment. The canals terminate in a layer of neuronal cell somata, which are connected to the brain by neurites (fig. 47a) and cells which stain brown. These cells are presumably glandular cells. The cerebral organ is in close contact to the blood vessels.

Eyes

Eyes are numerous and of the pigment cup type. The neuronal cell somata surrounding the posterior parts of the eyes represent type 1 described for the brain. The neurites of the sensory cells have contact to the neuropil of the brain (fig. 47a).

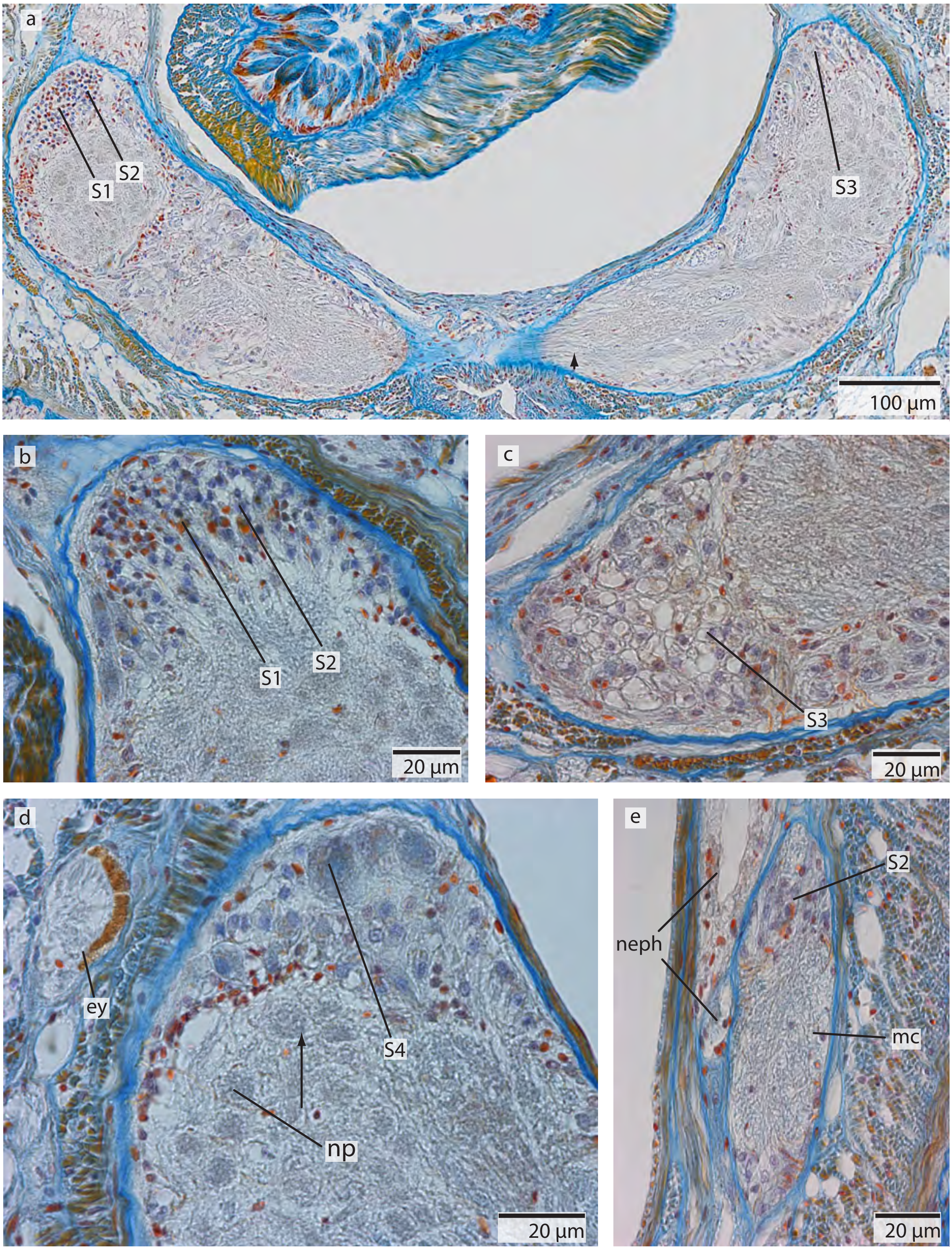


Fig. 48: *Amphiporus imparispinosus*, LM, cross sections, Azan **a**: Overview of the brain region showing the location of the different cell somata types (*S1- S3*). The neurites of the commissural plexus are horizontally arranged (*arrowhead*) **b**: higher magnifcation of *S1,S2* showing the cell somata type (*S1, S2*) which cell bodies are circular and they appear in cluster. Nuclei of *S1* dye *purple*, Nuclei of *S2 red*. **c**: Higher magnifcation of *S3* showing the cell somata type (*S3*) which nuclei dye also *purple* and are circular but the cell body is more prominent than in S1-S2. **d**: Cell bodies of somata cell type 4 (*S4*) are much more prominent than in S1- S3. The neuropil (*np*) of the brain form circular structures (arrow) in the dorsal brain regions. The eyes (*ey*) are in close contact to the brain. **e**: the cell somata of the medullary cords (*mc*) are of the S2 type. The medullary cords (*mc*) are in close contact to the nephridia (*neph*).

3.1.13 *Emplectonema gracile* (Emplectonematidae, Hoplonemertea)

Members of the *Emplectonematidae* are characterized by a round head which is fairly demarcated from the trunk. Several eyes are located on the margins of the head (fig. 6d). The brain is embedded in the head musculature.

Brain and central nervous system

The posterior part of the brain is divided into two lobes (fig. 49c). Both lobes are connected by commissural tracts. The ventral commissural tract arises prior the much smaller dorsal one (figs. 49b, c). The dorsal lobe ends blindly, in a layer of neuronal cell somata of type 2 and 3, in close proximity to the blood vessels separated by a thin layer of *ecm*. The ventral lobe is confluent with the lateral medullary cords. The lateral medullary cords run to the posterior of the animal embedded in a layer of longitudinal muscles. The neuronal cell somata cover the neuropil of the medullary cords in an u-shaped manner and cap the neuropil to the interior. The neuronal cell somata are of type 2 described for the brain (fig. 49d).

The brain of *Emplectonema gracile* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata (figs. 49b; 50a- c). There are at least 3 types of neuronal cell somata present (fig. 50a- c). The nuclei of the neuronal cell somata type 1 dye red and are primarily found on the lateral parts of the dorsal lobe of the brain (fig. 50a, b). The nuclei of type 2 neuronal cell somata dye bright purple and cell bodies are more prominent than in S1 (fig. 50b). Type 3 neuronal cell somata have larger diameter cell bodies than the latter and the cytoplasm dyes bright orange (fig. 50c). Type 1 and 2 neuronal cell somata are distributed all over the brain.

The central part of the brain neuropil is not separated by an inner neurilemma from the cell somata. The whole brain is surrounded by an outer neurilemma (fig. 50c). The neuropil of the brain shows different arrangements in different parts of the brain. In the ventral commissural tract they are horizontally arranged (fig. 50a). In the dorsal lobe of the brain the neurites of the neuronal cell somata form circular structures (fig. 50c). The neurites that connect the dorsal with the ventral part of the brain are vertically arranged (fig. 50b).

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *E. gracile* are very short and originate from the peripheral anterior margins of the dorsal part of the brain. The cephalic nerves are enclosed by a neurilemma. There is no layer of neuronal cell somata covering the neuropil of the cephalic nerves. The nerves are embedded in the musculature. There is no ventral or dorsal nerve present in *E. gracile*. The foregut nerves originate from the dorsal part of the lateral medullary cords. The number and origin of the proboscidial nerves could not be reconstructed.

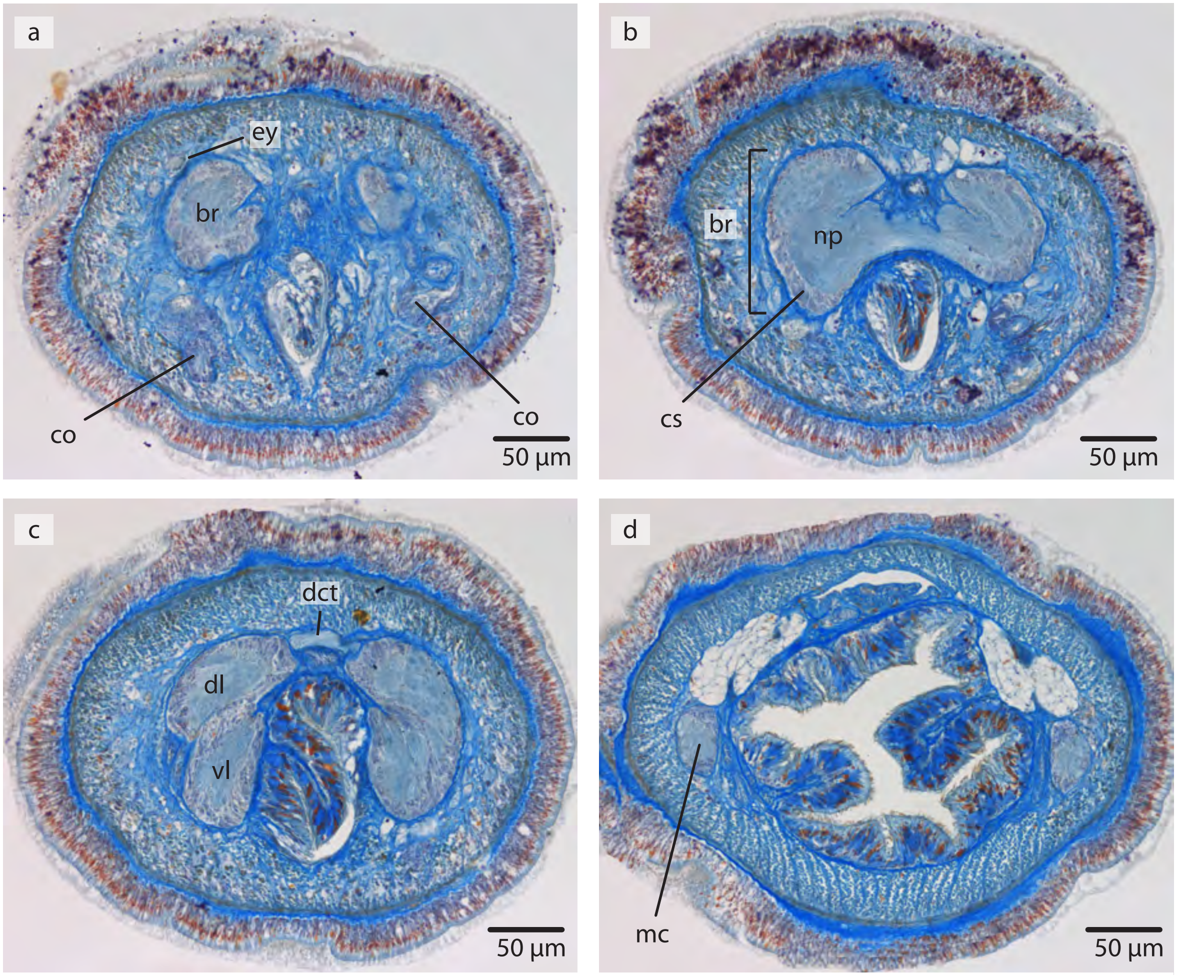


Fig. 49: *Emplectonema gracile*, LM, cross sections, Azan. **a**: The cerebral organs (*co*) and the eyes (*ey*) are in close contact to the anterior part of the brain (*br*). **b**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by cell somata (*cs*). **c**: In its posterior part the brain is divided into a dorsal (*dl*) and ventral (*vl*) lobe. The two parts of the brain are connected by a dorsal commissural tract (*dct*). **d**: The ventral lobes of the brain are confluent with the lateral medullary cords (*mc*).

Sensory structures

Frontal organ

A sensory organ is situated on the top of the animals head in front of the brain. It consists of two ducts that run to the inner of the animal but do end in front of the basal lamina of the epidermis.

Cerebral organ

The cerebral organ is located in anterior to the brain (fig. 49a). It consists of paired ducts which open ventrally to the exterior (fig. 49a). The ducts of the cerebral sense organ run to the inner of the animal and end in a layer of neuronal cell somata ventrally to the brain, close to the blood vessels. There is no direct connection of the cerebral organ to any part of the brain. The neuronal cell somata surrounding the duct are of type 2 described for the brain.

Eyes

Pigmented eyes are numerous in *E. gracile*. They are arranged on the margins of the head (fig. 6c). The eyes are of pigment cup type. There is a connection of the neurites of the eyes to the neuropil of the brain.

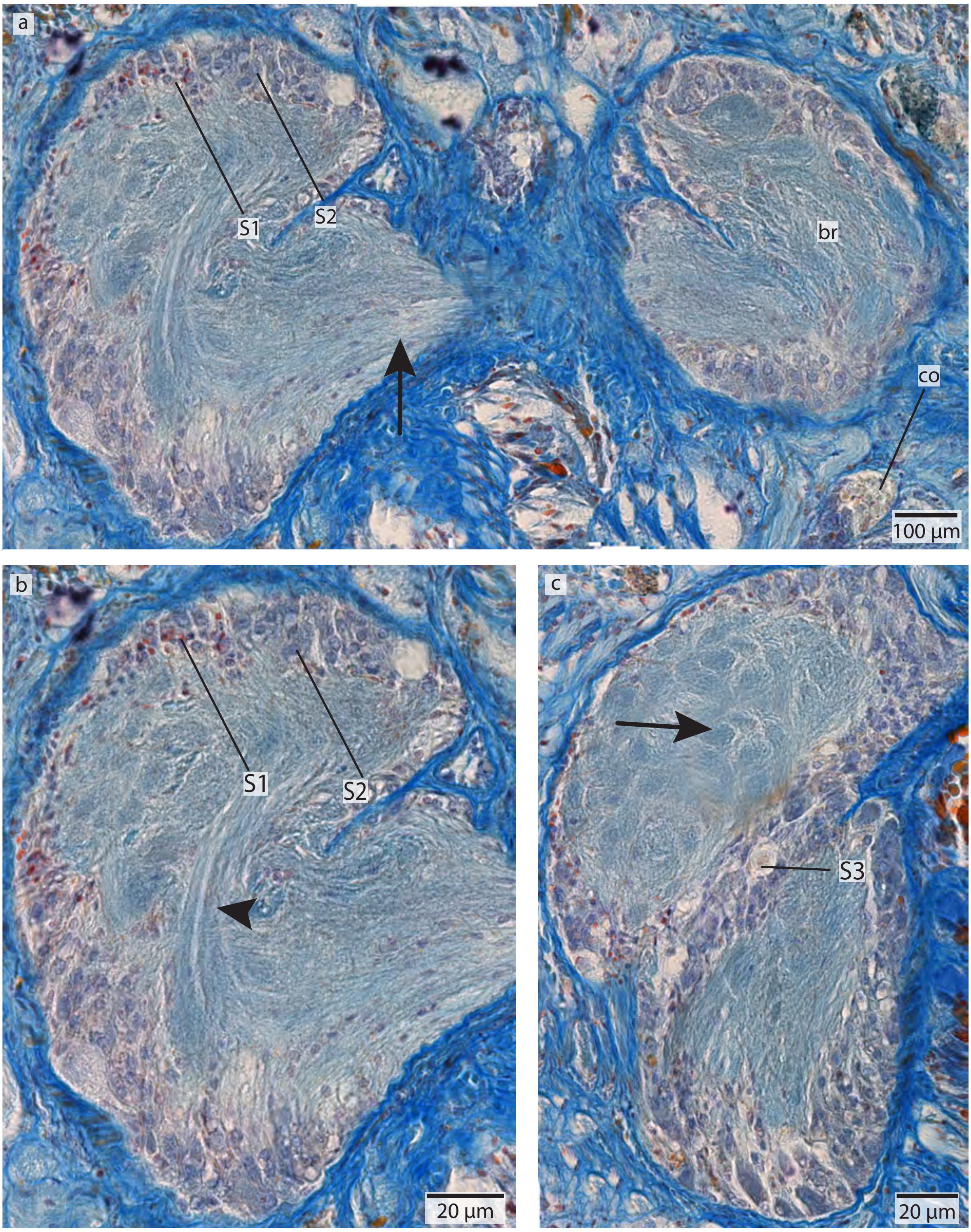


Fig. 50: *Emplectonema gracile*, LM, cross sections, Azan. **a**: overview of the brain (*br*) region showing the location of the different cell somata types 1- 3 (*S1 - S3*). The cerebral organ (*co*) is in close contact to the brain (*br*). The neurites of the ventral commussural tract are horizontally arranged (*arrow*). **b**: higher magnifcation of *S1*, *S2*. Nuclei of *S1* dye *red*, while nuclei of *S2* dye *purple*. The neurites (*arrowhead*) connecting the ventral with the dorsal part of the brain are vertically arranged. **c**: Nuclei of cell somata type 3 (*S3*) dye bright *orange*. The cell body is more prominent than in S*1 - S2*. The neurites (*arrows*) of the brain neuropil form circular structures in the dorsal brain region.

3.1.14 *Prosorhochmus claperedii* (Prosorhochmidae, Hoplonemertea)

Members of the *Prosorhochmidae* are characterized by a heart-like infold on the head. The brain is externally visible (fig. 6e) and embedded in the head musculature.

Brain and central nervous system

The posterior part of the brain is divided into a dorsal and ventral lobe (fig. 51d). The two parts of the brain are connected by a very small dorsal commissural tract and a posterior located more prominent ventral commissural tract. The ventral lobes are confluent with the lateral medullary cords; the dorsal lobe ends blindly in a layer of neuronal cell somata close to the blood vessels (fig. 51e). The medullary cords run underneath the outer longitudinal muscle layer . The neuronal cell somata of the medullary cord are not separated by *ecm* to the neuropil. The neuronal cell somata are of type 2 described for the brain and cap the medullary cords dorsal and ventral. The lateral medullary cords are in a close association with the nephridia (figs. 51f; 52c).

The brain of *P. claperedii* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata (fig. 51c). There are two different types of cell somata discernable (figs. 52a, b). The nucleoli of type 1 neuronal cell somata stain red (fig. 52b).

The nucleoli of type 2 neuronal cell somata stain dark purple and the cell bodies are enlarged compared to *S1* (fig. 52b). These cells are found on the periphery of dorsal lobes of the brain. The neuronal cell somata are not separated by an inner neurilemma from the neuropil of the brain, the whole brain is enclosed by an outer neurilemma (fig. 52a). The neuropil of the brain shows different arrangement of the neurites of the cell somata. In some parts especially in the commissural tracts, the neurites are horizontally arranged. In the dorsal lobe of the brain the neurites form cluster of circular structures (fig. 52a). The neurites connecting the ventral with the dorsal lobe of the brain are vertically arranged.

Minor nerves and peripheral nervous system

The numerous cephalic nerves are arranged in groups around the rhynchocoel (fig. 51a). They originate in the inner margins of the anterior part of the brain. No dorsal and ventral nerve are present. There are 12 proboscidial nerves present which are interconnected by circular nerves (fig. 51b).

Sensory structures

Cerebral organ

The cerebral organs of *Prosorhochmus claperedii* are situated in front of the brain (figs. 51a, b). They consist of small ducts which open to the environment ventro-lateral (figs. 51a, b). The ducts widen to small canals which ends in a layer of cell somata close to the blood vessels. The cell bodies of these cells are small and nuclei dye dark purple. They resemble the type 1 & 2 neuronal cell somata described for the brain. The cerebral organ is in its posterior part closely associated with the brain. It is not clearly discernable whether the brain neuropil has direct contact to the cerebral organ.

Eyes

P. claperedii possesses numerous pigmented eyes which are arranged on the margins of the head (figs. 6d; 51a). The eyes are of pigment cup type and are associated with the same cell somata type that is found associated with the cerebral organ and the brain (fig. 51a). That implies that these cells are receptor cells. The neuropil of the brain seems to be connected to the neurites of the eyes.

Cephalic gland

A cephalic gland is located dorsally around the rhynchocoel (fig. 51b) and extends posteriorly to the nephridia (fig. 51f)

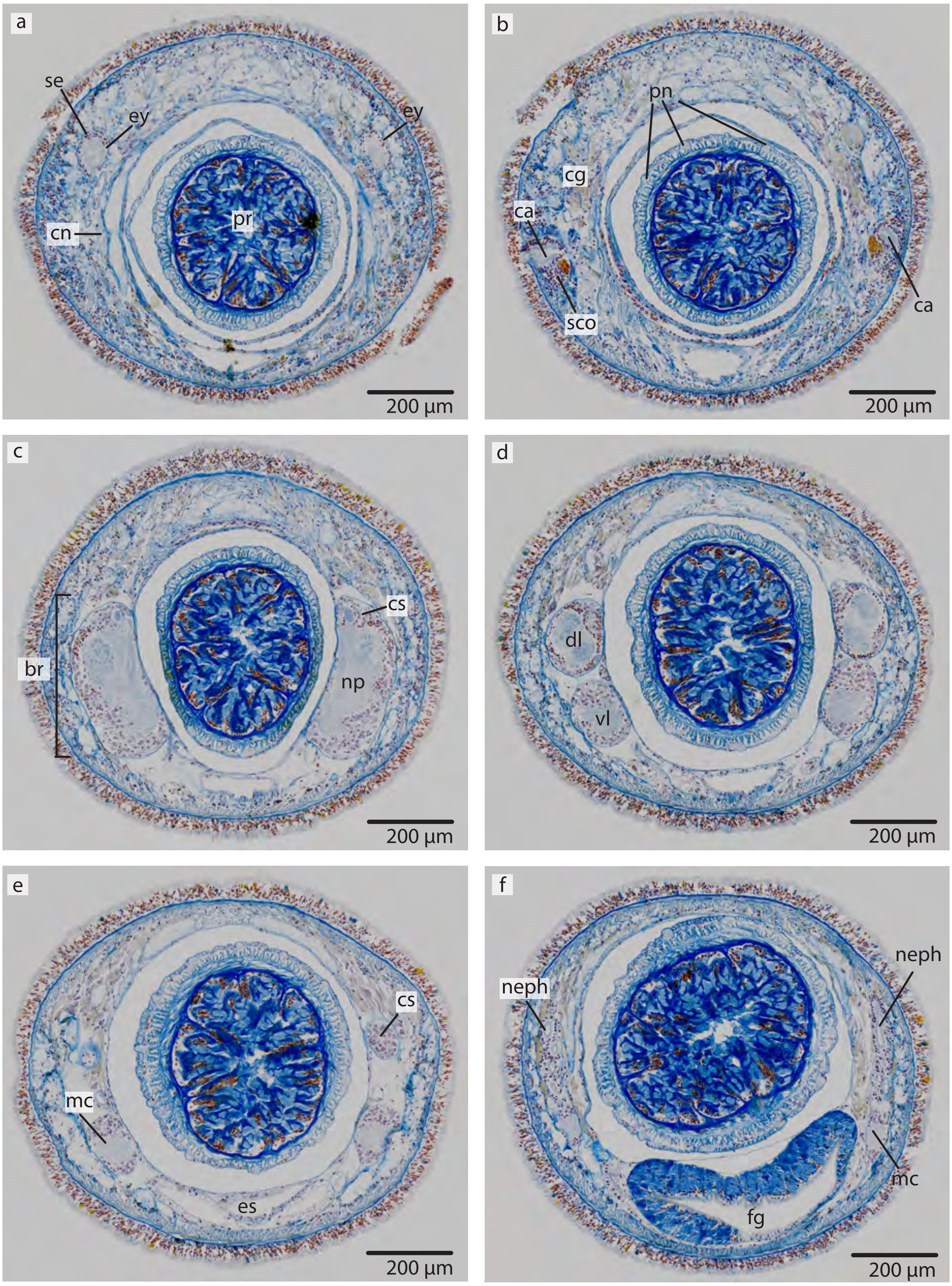


Fig. 51: *Prosorhochmus claperedii*, LM, cross sections, Azan. **a**: Frontal part of *P. claperedii* showing the cephalic nerves (*cn*), the eyes (*ey*) and the neuronal cell somata of the eyes (*cse*). **b**: The canals (*ca*) of the cerebral organs are surrounded by cell somata and open laterally to the environment. 12 proboscidal nerves (*pn*) are present. A cephalic gland (*cg*) is located dorsally. **c**: the two lobes of the brain are composed of a central neuropil (*np*) and the surrounding cell somata (**cs**). **d**: the brain divides posteriorly into a dorsal (*dl*) and ventral (*vl*) lobe. **e**: The lateral medullary cords (*mc*) extend from the ventral lobes to the posterior of the animal. The dorsal lobe ends in a layer of cells somata (*cs*). es: esophagus. **f**: The lateral medullary cords are in close association with the nephridia (*neph*). *fg* foregut.

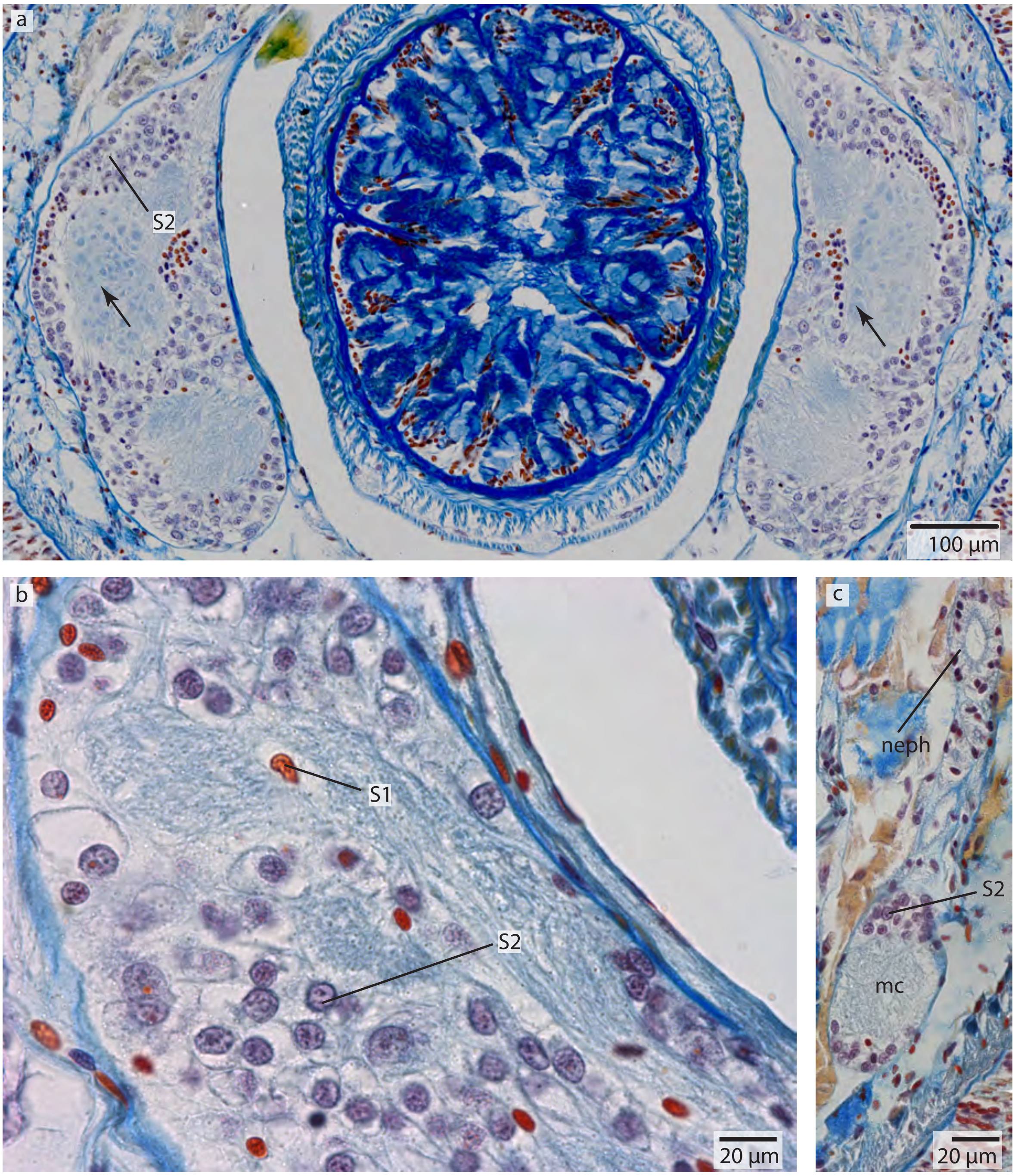


Fig. 52: LM, cross sections, Azan. **a**: Two types of neuronal cell somata are discernable (*S1*, *S2*). In the dorsal lobe of the brain the neurites form spherical structures (*arrow*). **b**: Higher magnifcation of *S1*, *S2*. Cell bodies of *s1* are not enlarged and the nuclei dye *red*. Cell bodies of cell somata type 2 are enlareged and nuclei dye *bright purple*. **c**: The lateral medullary cords (*mc*) are in close contact to the nephridia (*neph*). Somata of the medullary cord are of the 2 type described for the brain.

3.1.15 *Oerstedia dorsalis* (Prosorhochmidae, Hoplonemertea)

The head of *Oerstedia dorsalis* is not demarcated from the trunk. The brain of *O. dorsalis* is located in the head musculature. It is not externally visible (fig. 6f).

Brain and central nervous system

The very posterior part of the brain is divided into a dorsal and ventral lobe. The two halves of the brain are interconnected by a dorsal and ventral commissural tract. The dorsal commissural tract is much more prominent and lies anterior to the ventral commissural tract (fig. 53c). The dorsal lobe is bifurcated and the superior part ends blindly in a layer of neuronal cell somata close to the blood vessels. The inferior part gives rise to the accessory lateral nerve cord (fig. 53d). The ventral lobes give way to the lateral medullary cords. The medullary cords run underneath the longitudinal muscle layer. The neuropil of the medullary cord is not divided by an inner neurilemma from the neuronal cell somata. The neuronal cell somata cap the neuropil of the medullary cords dorsally and ventrally. The neuronal cell somata resemble the type 2 neuronal cell somata of the brain (fig. 53d). The medullary cords are in their anterior part closely associated with the nephridia (fig. 54c). An accessory lateral nerve cord is present and originates in the inferior dorsal lobe of the brain (fig. 53b).

The brain of *O. dorsalis* is composed of a central neuropil which is surrounded by neuronal cell somata (figs. 53c; 54a- c). There are at least two types of neuronal cell somata discernable (fig. 54a). Type one neuronal cell somata is small and slender and the nucleus stains bright purple (fig. 54b). This cell type is primarily found on the dorsal lobe of the brain. Cell bodies of type two neuronal cell somata are circular and enlarged compared to type 1 cell. The nucleus stains purple (fig. 54b). These cells are distributed all over the brain.

The central part of the brain neuropil is not divided by *ecm* to the neuronal somata. The whole brain is enclosed by an outer neurilemma (fig. 54b).

The neuropil of the brain shows a different arrangement of the neurites of the neuronal cell somata of the brain. In the commissural tracts the neurites are horizontally arranged. The neurites connecting the ventral with the dorsal lobe are vertically arranged. In the dorsal part of the brain the neurites form spherical structures (fig. 53c).

Minor nerves and peripheral nervous system

The numerous cephalic nerves originate from the inner margins of the anterior part of the brain.(fig. 53b). They are embedded in the longitudinal muscle layer. There are no neuronal cell somata around the neuropil of the cephalic nerves. The ten proboscidial nerves originate in the dorsal commissural tract (fig. 53b). No dorsal or ventral nerve is present in *O. dorsalis*.

Sensory structures

Frontal organ

In the very tip of the head is a dorsally located frontal organ (fig. 54d). It consists of a small pit which opens to the environment dorsally and a short canal which ends blindly in a layer of neuronal cell somata. No connection to the brain neuropil was observed.

Cerebral organ

The cerebral organ is situated in the tip of the animals head close to the first pair of eyes, but far away from the brain (fig. 53a). They gain contact to the environment through small pits which open ventrally (fig. 53a). The pits widen to small ciliated ducts which run to the interior of the animal and end in a neuronal cell somata layer. There was no close contact to the blood vessel discernable.

The neuronal cell somata are of 2 type described for the brain. The posterior part of the neuronal cell somata of the cerebral organ is adjacent to the neuropil of the eyes. It seems as if the neurites of the cerebral organ are in contact to the eyes.

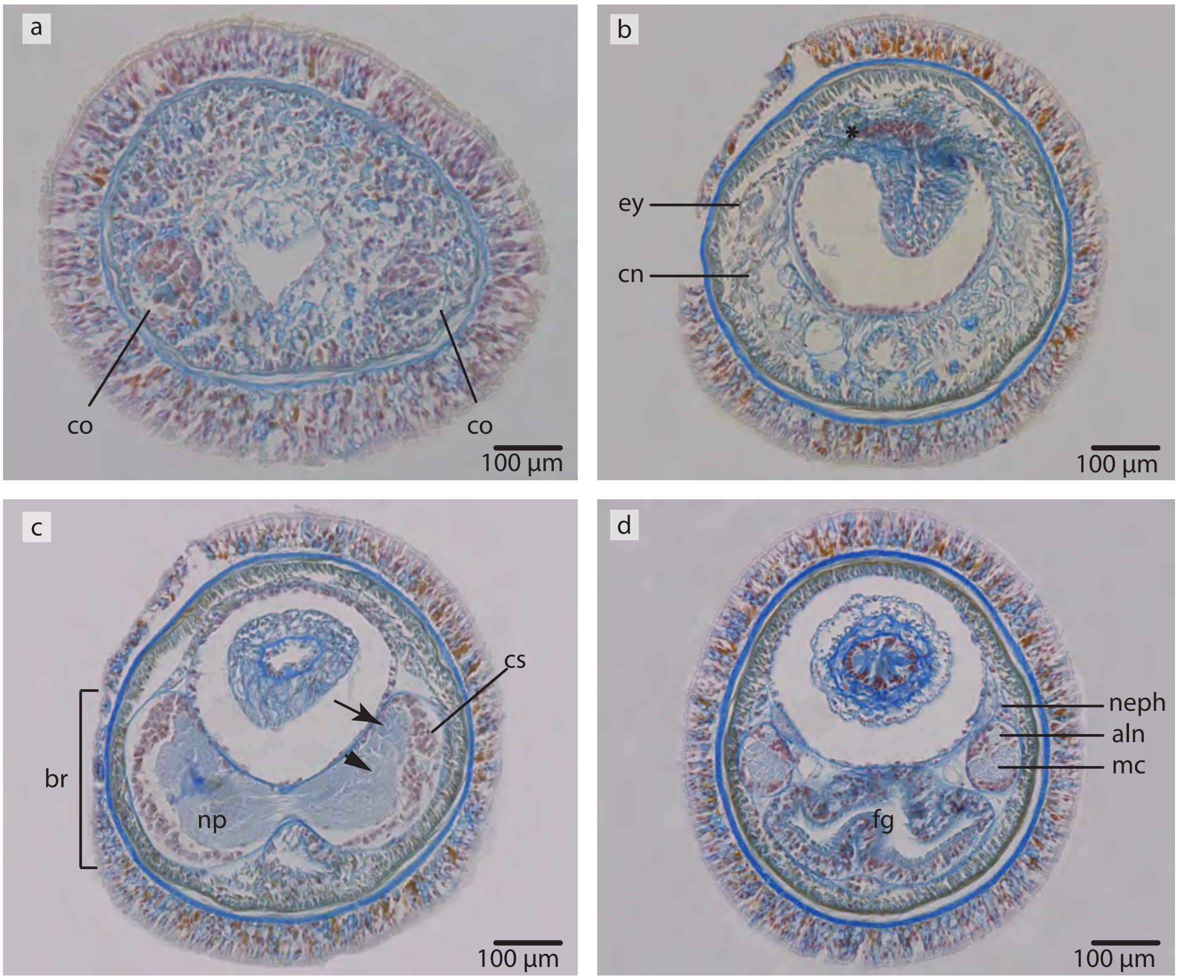


Fig. 53: *Oerstedia dorsalis*, LM, cross sections, Azan. **a**: Frontal part of *O. dorsalis* showing the cerebral organs (*co*) lying anterior to the brain. **b**: The second pair of eyes (*ey*) is in close contact to the cephalic nerves (*cn*). The proboscidial nerves originate in the dorsal commissural tract (*asterix*). **c**: The brain (*br*) is composed of a central neuropil (*np*) which is surrounded by neuronal cell somata (*cs*). The neurites in the dorsal part of the brain form circular structures (*arrow*). The neurites connecting the ventral with the dorsal lobe are vertically arranged (*arrowhead*). **d**: The lateral medullary cords (*mc*) are in close contact to the nephridia (*neph*). The accessory lateral nerve (*aln*) originates in the dorsal lobe of the brain. *fg* foregut.

Eyes

O. dorsalis possesses two pairs of eyes. The eyes are of pigment cup type (fig. 53b). The frontal pair is far anterior to the brain and there is no connection to the brain visible. The second pair of eyes is in close proximity to the frontal part of the brain. There is a connection between the eye neuropil and the neuropil of the brain. The neuronal cell somata found in the proximity of the eyes are of type 1 and are also found in the proximity of the cerebral organ.

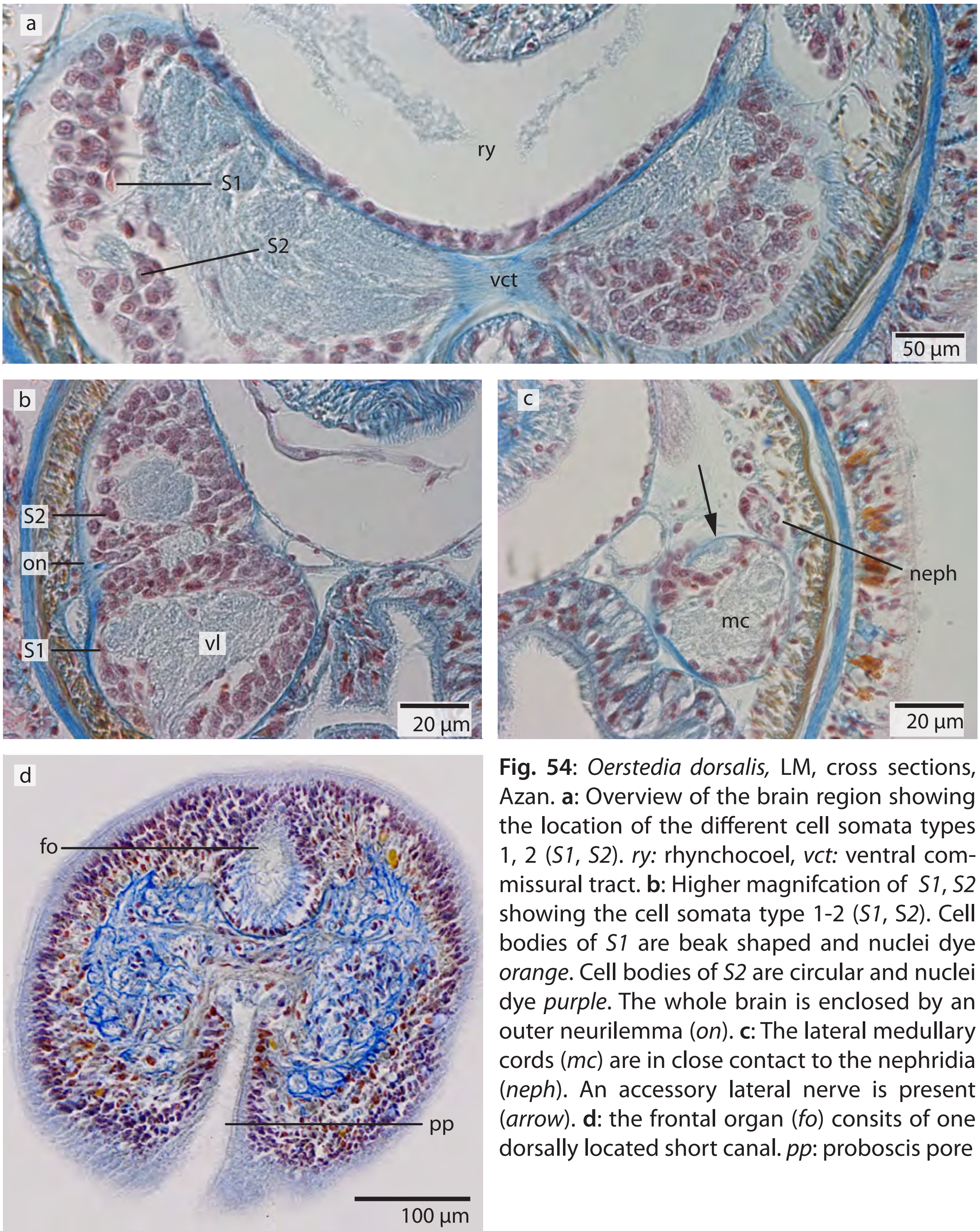


Fig. 54: *Oerstedia dorsalis,* LM, cross sections, Azan. **a**: Overview of the brain region showing the location of the different cell somata types 1, 2 (*S1*, *S2*). *ry:* rhynchocoel, *vct:* ventral commissural tract. **b**: Higher magnifcation of *S1*, *S2* showing the cell somata type 1-2 (*S1*, *S2*). Cell bodies of *S1* are beak shaped and nuclei dye *orange*. Cell bodies of *S2* are circular and nuclei dye *purple*. The whole brain is enclosed by an outer neurilemma (*on*). **c**: The lateral medullary cords (*mc*) are in close contact to the nephridia (*neph*). An accessory lateral nerve is present (*arrow*). **d**: the frontal organ (*fo*) consits of one dorsally located short canal. *pp*: proboscis pore

3.1.16 *Nemertopsis bivittata* (Amphiporidae, Hoplonemertea)

The brain of *Nemertopsis bivittata* is embedded in the head musculature. It is not externally visible (fig. 55a).

Brain and central nervous system

The posterior part of the brain is divided into two parts. The dorsal lobe ends blindly in a layer of neuronal cell somata close to the blood vessels, merely being separated by a small layer of *ecm*. The ventral lobes give way to the lateral medullary cords. The two halves of the brain are interconnected by a dorsal and ventral commissural tract. The dorsal appears prior to the ventral which is more prominent.

The lateral medullary cords are embedded in the body musculature. The neuronal cell somata of the lateral medullary cord resemble the type 2 neuronal cell somata of the brain. The cells are in a u-shaped manner wrapped around the neuropil of the medullary cords and cap them to the interior. The medullary cords are in a close association with the nephridial system (figs. 55b, 56e, f; 57c).

The brain of *N. bivittata* is composed of a central neuropil which is surrounded by a layer of neuronal cell somata (figs. 55b; 56c; 57a). There at least two types of neuronal cell somata discernable (figs. 57a, b). Type 1 cell somata are located lateral on the dorsal part of the brain. The nuclei stain in dark purple and the cell bodies are circular in shape (fig. 57b). Cell bodies of type 2 neuronal cell somata are also circular but the cytoplasm is enlarged and the nucleoli are smaller (fig. 57b). These cells are distributed all over the brain. The neuronal cell somata are not separated by an inner neurilemma from the neuropil. The whole brain is enclosed by an outer neurilemma (fig. 56c). The neurites of the neuropil of the brain show a different arrangement in various parts of the brain. In ventral commissural tract they are horizontally arranged. In the dorsal lobe of the brain the neurites form spherical structures. The neurites connecting the ventral with the dorsal lobe of the brain the are arranged in a vertical direction.

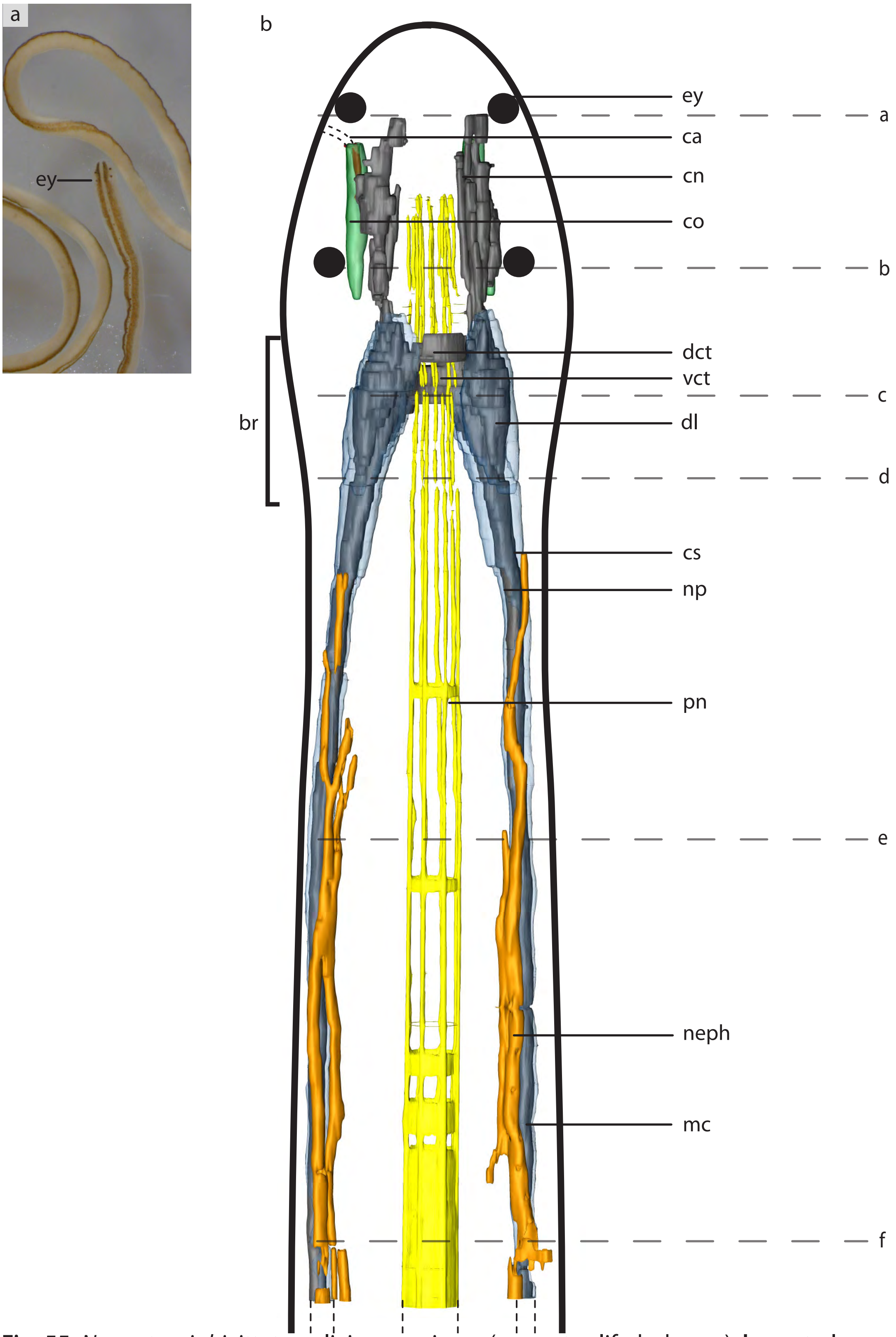


Fig. 55: *Nemertopsis bivittata*. **a**: living specimen (nemertea.lifedesks.org). **b**: central nervous system, dorsal view, snapshot 3d-reconstruction (143 slices). The nervous system is composed of neuropil (*np*, *gray*) which may be surrounded by cell somata (*cs*, *blue*). Cephalic nerves (*cn*) are arranged around the rhynchocoel, 8 proboscidial nerves (*pn*, *yellow*) are present. The dorsal lobes (*dl*) of the brain (*br*) are connected by a dorsal (*dct*) and the ventral by a ventral commissural (*vct*) tract, respectively. The canals (*ca*) of the cerebral organ (*co*) opens to the environment ventral. The lateral medullary cords (*mc*) are in close contact to the nephridia (*neph*). Letters on the right (**a** - **f**) refer to the histological sections in figure 56.

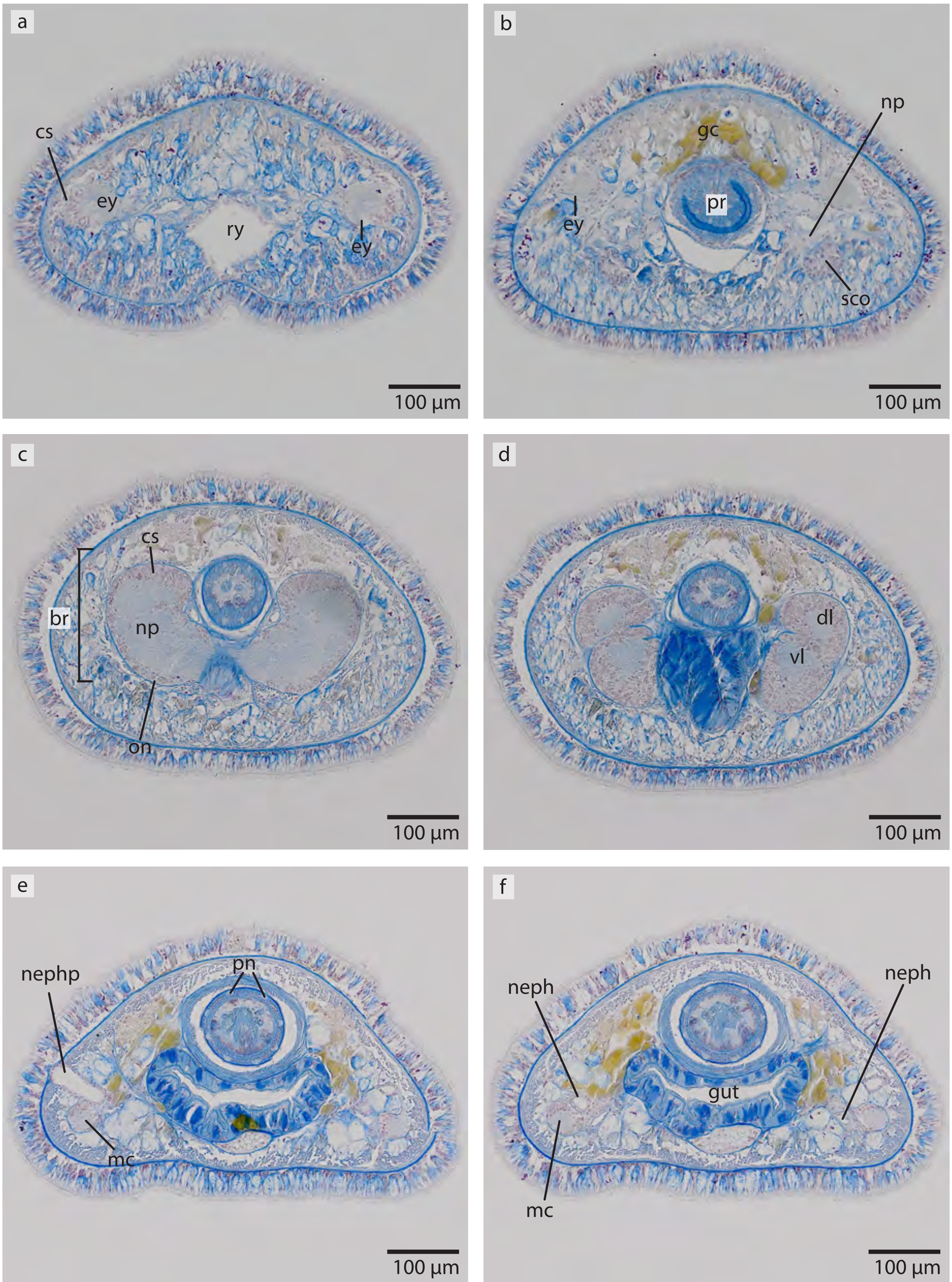


Fig. 56: *Nemertopsis bivittata*, LM, cross sections, Azan. **a**: frontal region showing the eyes (*ey*) and the associated cell somata (*cs*). *ry*: rhynchocoel. **b**: gland cells (*gc*) are located dorsally. The cerebral organs end in a layer of cell somata belonging to the cerebral organ (*sco*). The second pair of eyes (ey) is connected to the neuropil (*np*) of the brain. **c**: The brain is composed of a central neuropil (*np*) with its surrounding cell somata (*cs*). The whole brain is enclosed by an outer neurilemma (*on*). **d**: Posterior the brain divides into a dorsal (*dl*) and ventral lobe (*vl*). **e**: There are 8 proboscidial nerves (*pn*) present. A enormous nephridioporus (*nephp*) is located posterior to the brain. **f**: The lateral medullary cords (*mc*) are closely associated with the nephridia (*neph*). *pr* proboscis, *ry* rhynchocoel

Minor nerves and peripheral nervous system

The numerous cephalic nerves of *N. bivittata* originate from the lateral parts of the brain (figs. 55b, 56b) and are arranged in groups around the rhynchocoel. They are not covered by neuronal somata. There is no dorsal and ventral nerve in *N. bivittata*. Eight proboscidial nerves which are circular arranged around the margins of the proboscis are present (fig. 56e). The nerves are connected by circular nerves. The origin of the proboscidial nerves as well as the origin of the esophageal nerves could not be reconstructed.

Sensory structures

Cephalic glands

Cephalic glands arise shortly posterior to the second pair of eyes. They are located dorsally and are present to the midgut (figs. 56b- f).

Cerebral organ

The cerebral organ consists of small paired pits which open ventrally. The pits widen to short ciliated ducts which end in close proximity anterior to the brain in a layer of neuronal cell somata (fig. 55b). The neurites of the brain neuropil are connected to the neuronal cells somata. The neuronal somata cells here are of the 2 type described for the brain (fig. 56b).

Eyes

The eyes are of pigment cup type. The two pairs of eyes of *N. bivittata* are arranged on the margins of the animals 'head (figs. 55a, b; 56a, b). The first pair of eyes lies anterior to the brain. A connection of these eyes to the brain was not clearly discernable. The neuronal cell somata that are associated with the eyes resemble those of the cerebral organ (type 2). The neuropil of the brain is connected to the receptor cells of the second pair of eyes which is located on the same level with the brain (fig. 56b).

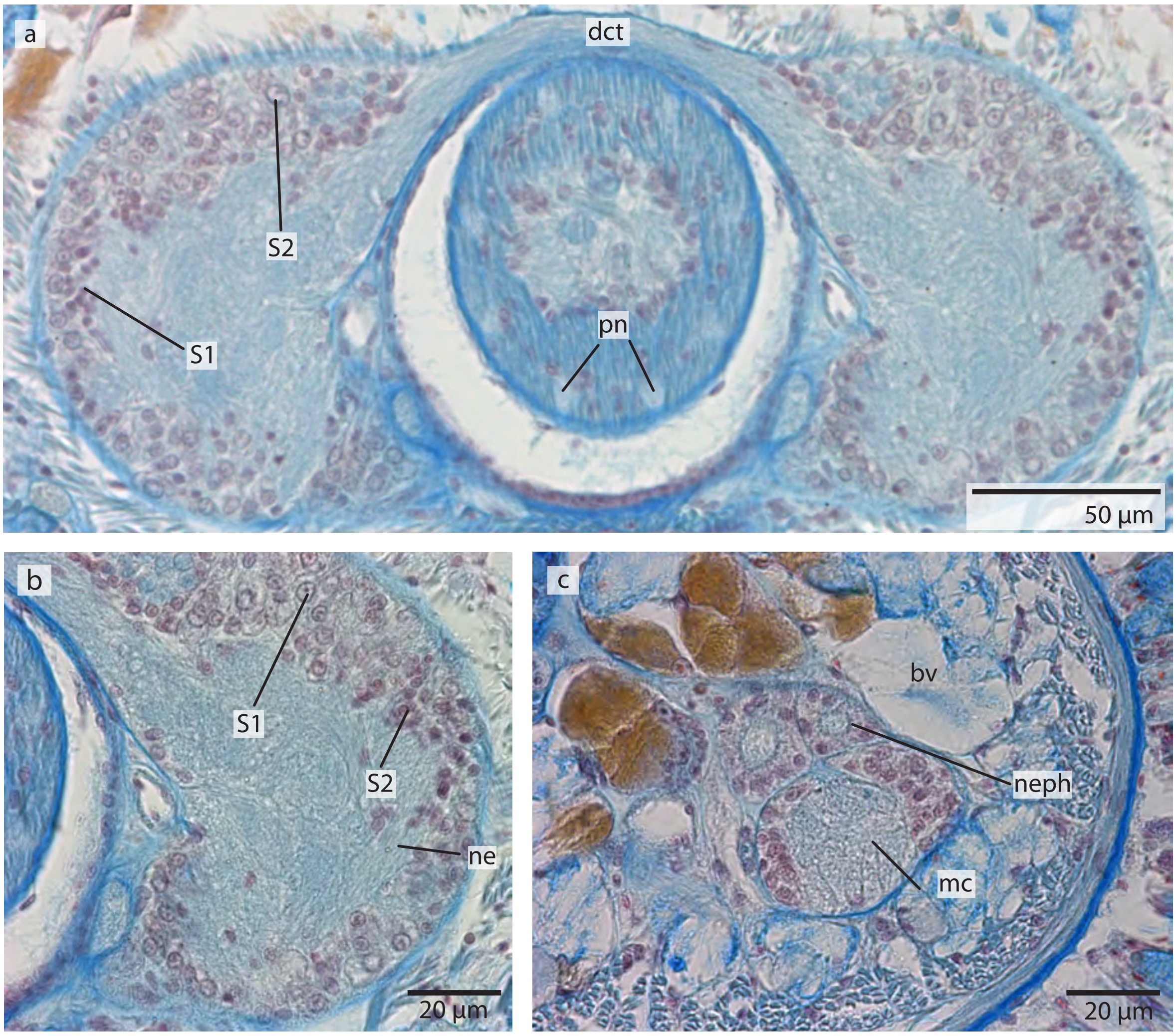


Fig. 57: *Nemertopsis bivittata*, LM, cross sections, Azan. a: overview of the brain region showing the location of the different cell somata types (*S1, S2*), *dct*: dorsal commissural tract, *pn*: proboscis nerve. b: higher magnifcation of *S1,S2* showing the cell somata type (*S1,S2*). Nuclei of *S1* and *S2* dye in purple. Cell bodies of neuronal cell somata type 1 are enlarged. The neurites (*ne*) of the brain neuropil are vertically arranged. **c**: the lateral medullary cords (*mc*) are in close contact to the nephridia (*neph*). *bv*: blood vessel.

3.2 Phylogenetic part

A total number of 61 morphological elements arose of this investigation. The implicit enumeration analysis resulted in 20 equally most parsimonious trees (L= 74). Using implied weighting the analysis resulted in most parsimonious 1 tree (L= 74; Ci= 72, Ri= 84). Changing the K score from 0.5 to 6 did neither change the topology of the tree nor the number of equally most parsimonious tress. The tree shows three major clades: Palaeo- Hetero- and Hoplonemerteans (fig.58).

Because palaeonemerteans are supposed to be the most basal member of the nemerteans in most studies (e.g. Bürger 1895, Gibson 1972, Thollesen 2003, Andrade 2011) members of this group were used to root the trees. As trees were rooted with different palaeonemertean taxa the analysis resulted in five strict consensus trees. The topology and bootstrap values of neonemertea and hetero- hoplonemertea do not change neither do the sistergroup relationships between *Carinina ochracea* and Cephalothricidae and *Callinera monensis* and *Tubulanus*. Tubulanid nemerteans (sensu stricto, comprising *Tubulanus*, *Callinera* and *Carinina* (Gibson 1972) are not monophyletic in any tree.

Two traditional hypotheses exist concerning the possible basal member of nemerteans: Bürger (1895) supposed *Carinina* to be the first basal most branching taxon of all nemerteans because of the intraepidermal located nervous system. He supposed an inward migration of the nervous system in the other “higher” nemertean taxa.

Iwata (1960) postulated that the condition found in Cephalothricidae (which he named Archinemertea) represents the ground pattern of nemerteans, namely the intramuscular location of the nervous system and the lack of a cerebral organ in Cephalothricidae as well as other morphological and embryological characteristics.

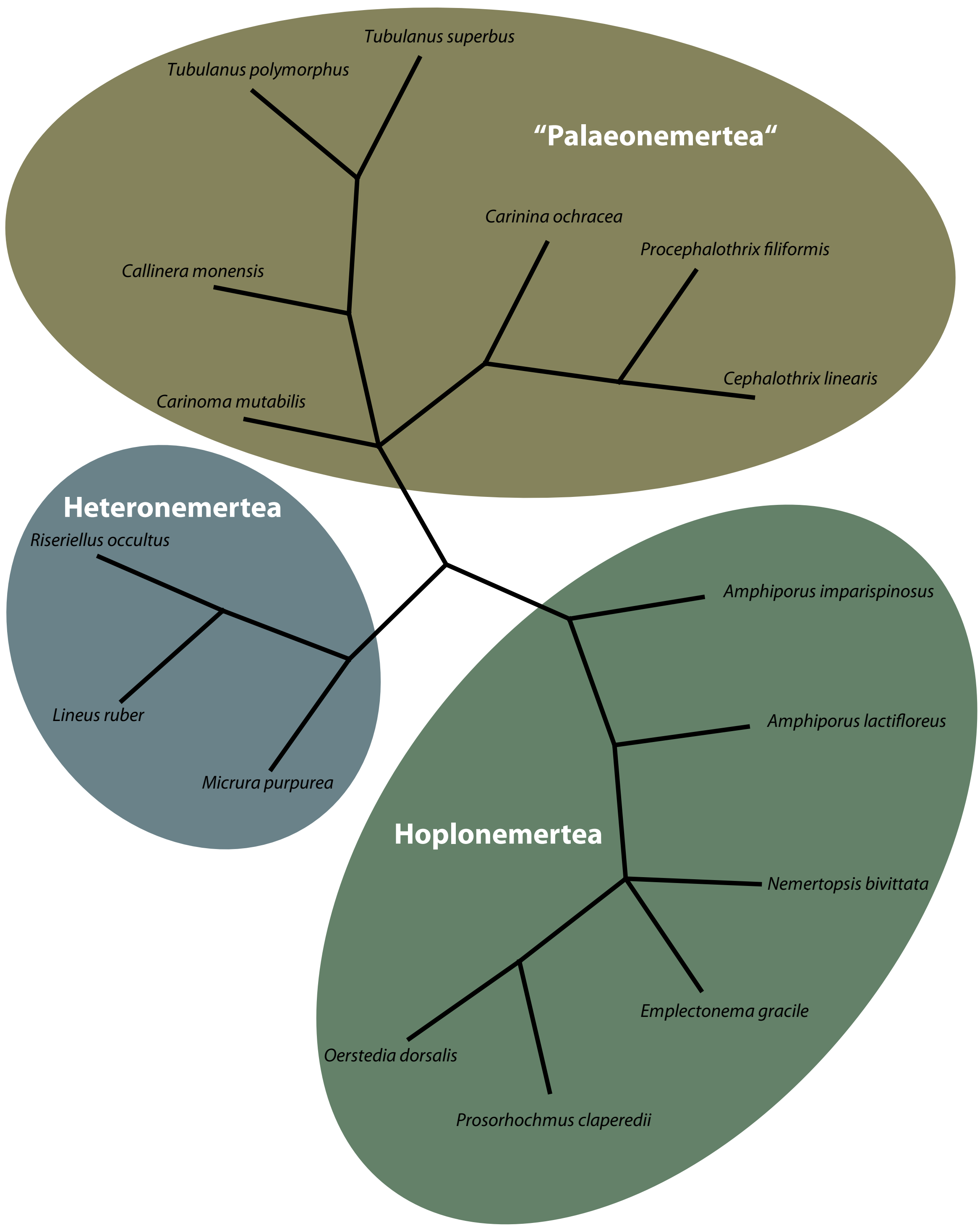


Fig. 58: Strict consensus tree (implicid eunimeration, implight weighting (K= 3.0)), unrooted. Three major clades are discernable: Palaeo- , hetero-, and hoplonemerteans.

If the tree is rooted with Cephalothricidae the resulting strict consensus tree shows a very good support for a group of nemerteans possessing a cerebral organ (Bootstrap value: BS: 99%). The monophyly of Hoplonemerteans (BS 99%) and Heteronemerteans (BS 92%) is well supported.

The group of Hetero- and Hoplonemertea (Neonemertea sensu Thollesson & Norenburg 2003) show a weak support (BS 44%). The relationships between *C. mutabilis*, *Tubulanus* and Heteronemerteans are not resolved. Tubulanid nemerteans sensu stricto are also not monophyletic, *Tubulanus* and *Callinera monensis* being closer related to heteronemerteans than to the remaining palaeonemerteans (fig. 59).

If the tree is rooted with *Callinera monensis* or *Carinoma mutabilis*, Cephalothricidae become the sistergroup of *Carinina ochracea* with weak support (BS 10). Cephalothricidae are monophyletic (BS 99) and together with *C. monensis* and *C. mutabilis* they are closer related to heteronemerteans than to the *Tubulanus*+ *Callinera monensis* group.

A sister taxon relationship between Cephalothricidae and *Carinina ochracea* is not likely. This relationship is may be due to long branch effects because of the visible morphological differences present between these two taxa (fig. 60).

If the cerebral organ was present in the ground pattern of nemerteans (*Carinina, Tubulanus*) it would have been reduced three times in *C. monensis*, *C. mutabilis* and the Cephalothricidae. Rooting the tree with *Carinina ochracea* results in early branching Cephalothricidae as the sister group to a taxon composed of *C. mutabilis, Tubulanus* + *C. monensis* and Neonemertea (sensu Thollesen & Norenburg 2003). Due to the two existing hypothesis concerning the basal taxon of nemerteans, the trees rooted with Cephalothricidae and *Carinina ochracea* respectively are presented here and discussed (figs. 59; 60).

In order to compare the present data with already published results the hypothesis published by Andrade et al (2011) was used to map the characters from the present matrix (winclada Nixon 1999). The resulting tree (L= 79) shows more evolutionary changes than the present analysis and is about 10 % longer. Since the only taxon missing in the research by Andrade et al. 2011 was *Oerstedia dorsalis*, the animal and the character accessory lateral nerve, which is only present in *O. dorsalis*) were neglected and the tree was recalculated. The implicit enumeration analysis without *O. dorsalis* resulted in 5 most parsimonious trees (L= 72). Using implied weighting (K= 3.0) the analysis resulted in 1 tree (L= 72; Ci= 73, Ri= 83). Neglecting *Oerstedia dorsalis* did not change the topology of the trees but their length. Hetero- and hoplonemerteans are again monophyletic and palaeonemerteans are not. The strict consensus tree shows the same overall topology as *O. dorsalis* included.

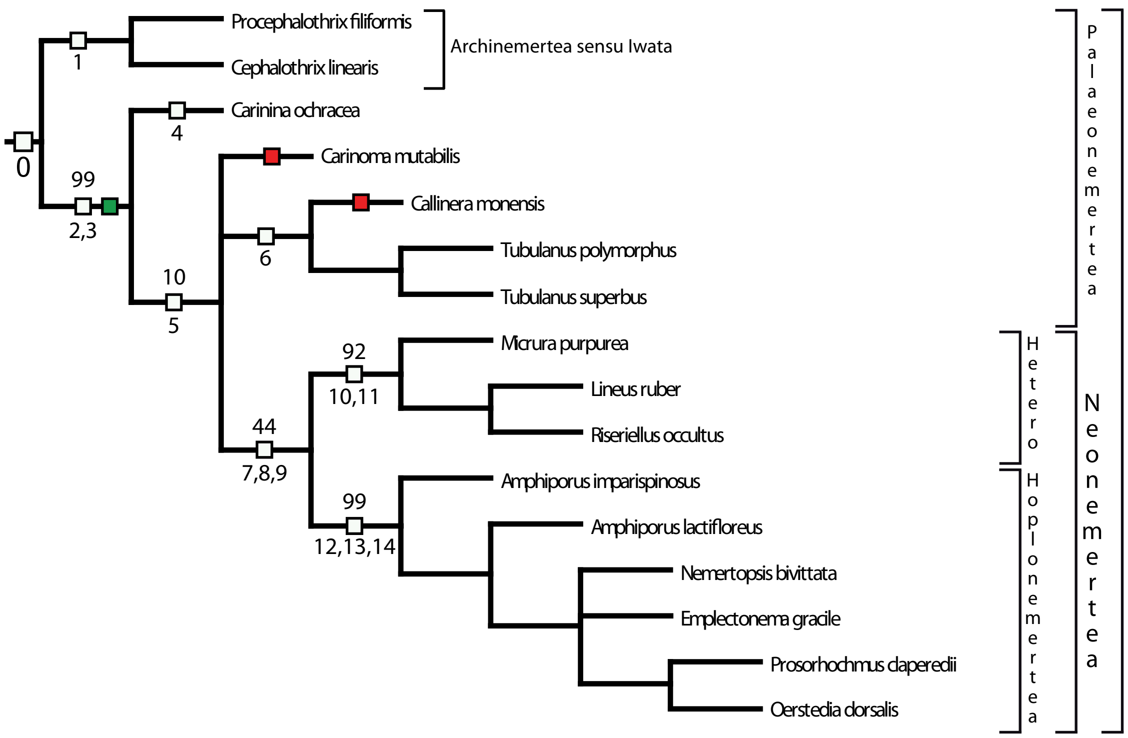


Fig. 59: Phylogeny based on characters of the nervous system using Cephalothricidae as out group. Strict consensus tree (implicid eunimeration, implied weighting (K=3.0)), bootstrap values above squares (1000 repl., implicid weighting, implicid eunimeration). *Number below squares*: unambigious characters supporting node. *red squares*: reduction of the cerebral organ. *green square*: origin of cerebral organ. *0*: nervous system inside musculature (pleisiomorphic), two lobed brain surrounding rhynchocoel,- daeum. The brain is composed of an inner neuropil with surrounding cell somata. Two lateral medullary cords originate in the ventral lobe of the brain. Two proboscidial nerves are present. A dorsal and ventral nerve originate in the dorsal or ventral commissural tract. Various nerve plexus are present in different parts of the body. *1*: cephalic nerve cords are solitary arranged. *2*: cerebral organ composed of a canal lined with sensory cells which are innervated by neurites of the brain showing immunoreactivity against α-tubulin. *3*: cephalic nerves arranged in groups as a closed circle around head lacuna. *4*: brain epidermal. *5*: outer neurilemma clearly discernable. *6*: brain directly below basal lamina of epidermis. *7*: canal of cerebral organ pierces basal lamina of epidermis. *8*: canal of cerebral organ ends in a special cell layer composed of cell somata and partially glandular cells. *9*: cephalic nerves in groups around rhynchocoel. *10*: brain tripartated. *11*: cerebral organ attached to the inferior dorsal lobe of brain. *12*: medullary cords in close contact to nephridia. *13*: dorsal lobe of brain ends close to blood vessel. *14*: more than two proboscidial nerves.

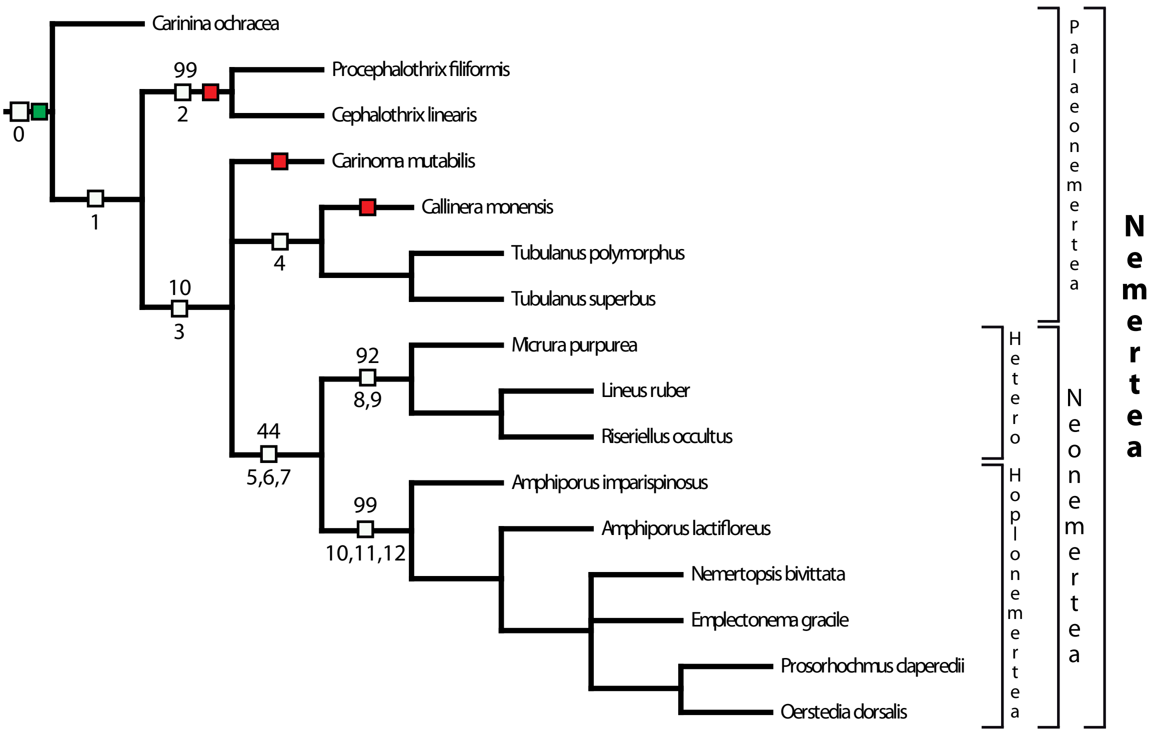


Fig. 60: Phylogeny based on characters of the nervous system using *Carinina ochracea* as out group species. Strict consensus tree (implicid eunimeration, implied weighting (K=3.0)), bootstrap values above squares (1000 repl., implicid weighting, implicid eunimeration). *number below squares*: unambigious characters supporting node. *red squares*: reduction of the cerebral organ. *green squares*: origin of the cerebral organ. *0*: nervous system intraepidermal, cephalic nerves circular arranged around head lacuna, two lobed brain surrounding rhynchocoel,- daeum. The brain is composed of an inner neuropil with surrounding neuronal cell somata. Two lateral medullary cords originate in the ventral lobe of the brain. Two proboscidial nerves are present. A cerebral organ composed of a canal lined with sensory cells which are innervated by neurites of the brain showing immunoreactivity against α-tubulin is present. A dorsal nerve originates in the dorsal commissural tract. Various nerve plexus are present in different parts of the body. *1*: brain inside musculature. *2*: cephalic nerve cords are solitary arranged. *3*: outer neurilemma clearly discernable. *4*: brain directly below basal lamina of epidermis. *5*: canal of cerebral organ passes basal lamina of epidermis. *6*: canal of cerebral organ ends in a special cell layer composed of cell somata and partially glandular cells. *7*: cephalic nerves in groups around rhynchocoel. *8*: brain tripartated. *9*: cerebral organ attached to the inferior dorsal lobe of brain. *10*: medullary cords in close contact to nephridia. *11*: dorsal lobe of brain ends close to blood vessel. *12*: more than two proboscidial nerves.

4 Discussion

4.1 Comparative anatomy

Due to the fact that nearly all nemerteans are hunters, their nervous system appears quite uniform in being composed of a four lobed brain and two lateral medullary cords. But the fine structure of nemertean nervous system differs considerably between evolutionary lineages and sometimes closely related species.

4.1.1 Brain and central nervous system

The brain is in general composed of a central neuropil with its surrounding neuronal cell somata. In palaeo- and heteronemerteans the neurites of the brain neuropil are more or less homogeneously arranged while they are arranged in different ways in various brain parts of hoplonemertea. The neurites of the commissural plexus are horizontally arranged in all investigated nemerteans. Only in hoplonemerteans the neurites in the brain parts which connect the ventral with the dorsal lobe of the brain are additionally vertically arranged. In the dorsal lobe the neurites form spherical structures which could be revealed with Azan staining but not with immunohistochemical methods.

The neuronal cell somata surrounding the brain neuropil can only be homologized in lineid heteronemerteans. Bürger (1895) described four, Bianchi (1969 a, b) six types of neuronal cell somata in *Cerebratulus marginatus* (Heteronemertea). Only neuronal cell somata of type 1- 3 described by Bürger for *C. marginatus* were found in heteronemerteans in the present study.

Neuronal cell somata in the remaining described nemerteans differ considerably in their staining affinities and morphology in even closely related species (e.g. Cephalothricidae). The coloration of the nuclei reaches from red to orange to purple. The cell bodies are in some cases enlarged (especially Heteronemertea). The cells may appear beak shaped (e.g. *Procephalothrix filiformis*), pear shaped (e.g. *Callinera monensis*) or circular (e.g. *Amphiporus*).

At the moment it is not possible to homologize the different neuronal cell somata and thus they were not considered for the phylogenetic analysis. The neuronal cell somata of the brain may be separated from the neuropil by an extracellular matrix, which was called inner neurilemma (Bürger 1895). In Cephalothricidae, *Carinina ochracea* and heteronemerteans an inner neurilemma is present while it is lacking in hoplonemerteans and *Callinera monensis* + *Tubulanus*. The whole brain may be enclosed by an outer neurilemma. The outer neurilemma is not clearly discernable in Cephalothricidae and *Carinina ochracea*.

Little is known about the function of the extracellular matrix in the invertebrate nervous system. The *ecm* is supposed to be involved in neurite formation, to maintain the structure and function, and in repairing processes (Rutka et al. 1988, Harrel & Tanzer 1993). The *ecm* surrounding the nervous system may also function as a barrier to shield the chemical activities in the nervous system.

Lateral medullary cords are present in all nemerteans. The location of the neuronal cell somata surrounding the medullary cords differ in their arrangement. With a few exceptions the somata cap the neuropil of the medullary cord dorsally and ventrally (e.g. palaeonemerteans). In some nemerteans the somata enclose the neuropil in an u-shaped fashion (*Amphiporus lactifloreus*, *Lineus ruber*). In hoplonemerteans the medullary cords are in close association with the nephridia. This arrangement is probably due to osmoregulatory or neurosecretory constrains.

4.2.2 Minor nerves and peripheral nervous system

Cephalic nerves differ in their arrangement and position between higher taxa. In Cephalothricidae there are four solitary arranged cephalic nerves. In the remainder of the palaeonemerteans (*Tubulanus*, *Callinera monensis*, *Carinoma mutabilis*, *Carinina ochracea*) they are circular arranged around the head lacuna. In hoplo- and heteronemerteans they are grouped but not circular arranged. Additionally only in Cephalothricidae the cephalic nerves are covered by a huge layer of neuronal cell somata. This condition characterizes the cephalic nerves of Cephalothricidae as frontal nerve cords (medullary cords).

A dorsal nerve is present in palaeo- and heteronemerteans but absent in investigated hoplonemerteans. The dorsal nerve of Cephalothricidae and *Carinoma mutabilis* originates in the dorsal commissural tract inside the musculature. In the further course the nerve "migrates" dorsally and runs finally just underneath the basal lamina of the epidermis (subepidermal). A ventral nerve is only present in Cephalothricidae. This nerve gives rise to the esophageal nerves at the level of the mouth opening. Posterior to the mouth opening the paired esophageal nerves fuse again and give rise to the continuing ventral nerve.

Esophageal nerves are always present and differ in their origin between higher taxa. In palaeonemerteans they originate in the ventral commissural tract of the brain. In heteronemerteans they arise from the ventral lobes of the brain. The origin of the esophageal nerves of hoplonemerteans could only be reconstructed in *Emplectonema gracile and Amphiporus lactifloreus*. Here they also arise from the ventral lobes of the brain.

Investigated palaeo- and heteronemerteans possess paired proboscidial nerves which originate in the ventral commissural tract of the brain. In hoplonemerteans the number of proboscis nerves may rise up to 16 in *Amphiporus*. The proboscis nerves originate in the dorsal commissural tract in *Amphioprus lactifloreus*, *Oerstedia dorsalis*, *Emplectonema gracile* and *Nemertopsis bivitatta*. In the remainder of hoplonemerteans the origin could not be reconstructed.

Nemerteans possess nerve plexus in different parts of their body. These plexus may be well developed and are present in all immunohistochemical investigated taxa. The arrangement of the neurites there differ between nerve plexus but not between species. The neurites of the intraepidermal nerve plexus are arranged in a regular, ladder like manner. The subepidermal plexus may be well developed and neurites there are parallel arranged. The neurites of the subrastomatogastric, rhynchocoelan nerve plexus and of the commissural plexus are also parallel arranged. The neurites of the intrastomatogastric nerve plexus are arranged in an irregular, net like manner. The proboscidial plexus connects the main proboscidial nerves. The different plexus are in some cases interconnected by fine branches of neurites.

4.1.3 Sensory structures

Nemerteans perceive chemical substances with different chemo sensitive sensory structures which are situated on the head of the animals. These are called the frontal and cerebral organs. As it is shown by Wang & Sun (2006) and Amerongen & Chia (1983) these sensory structures are designed for a hunting life style. Frontal organs are only present in investigated hetero- and hoplonemerteans species, although they have been described for *Procephalothrix simulus* (Palaeonemertea) (Iwata 1952). Frontal organs are very small canals which appear quite similar to the canals of the cerebral organ. But in contrast to the canals of the cerebral organs no neurites of sensory cells connected to the brain showing immunoreactivity against α-tubulin are found there. In *Lineus ruber* and *Riseriellus occultus* (Heteronemertea) the frontal organ consist of one dorsal and two lateral pits, while in *Oerstedia dorsalis* (Hoplonemertea) it consists of one dorsally located pit.

The cerebral organs of *Tubulanus* consists of simple epidermal tubes which open laterally to the environment and terminate exterior to the basal lamina in the epidermis. The inner part of the epithelium of the tube is lined with sensory cells which are connected to the brain. In *Carinina ochracea* the canal of the cerebral organ is elongated and runs dorsally but also terminates exterior to the basal lamina of the epidermis. In contrast to the conditions found in *Tubulanus* the duct of the cerebral organ of *C. ochracea* passes through the neuronal cell somata of the brain due to the fact that the brain in *C. ochracea* is intraepidermal.

The cerebral organ of heteronemertean species is composed of an epidermal canal and a convoluted duct which terminates in a layer of glandular cell and neuronal cell somata. The duct runs below the basal lamina of the epidermis. The cerebral organ is attached to the inferior dorsal lobe of the brain and located close to the blood vessels.

The cerebral organs of hoplonemerteans are in most cases located anterior to the brain. The duct also terminates in a layer of neuronal cell somata and glandular cells close to the blood vessels. The duct is not convoluted.

Pigmented eyes are present in all adult investigated Neonemertea, except *Micrura purpurea*, but absent in investigated adult Palaeonemertea. The eyes are of pigment cup type. A shielding pigment is present and ciliated sensory cells of the eyes proceed into the optical cavity and are connected to the central nervous system (Jespersen & Lützen 1988, von Döhren & Bartolomaeus 2007). Only in *Amphiporus imparispinosus* and *Nemertopsis bivittata* a connection of the sensory cells of the eyes to the brain was visible in Azan staining but not with immunohistochemical methods. The neurites connecting the sensory cells of the eyes to the brain are presumably not immunoreactive against FMRF, serotonin and α-tubulin.

In *Carinina ochracea* several sensory cells originate in the subepidermal plexus and proceed into the outer margins of the epidermis. These cells may have a tactile function. In *Procephalothrix filiformis* a cluster of cells showing immunoreactivity against FMRF is present in the tip of the animal´s head. These cells might represent sensory cells.

4.2 Characters and character coding

A total number of 61 morphological elements concerning the nervous system arose of this investigation. Characters and explanations are listed below. Morphological data matrixes are already available for nemerteans (e.g. Sundberg & Hylbom 1994, Sundberg et al. 2003, Schwartz and Norenburg 2001), but all of them primarily deal with other morphological elements than those of the nervous system. The analysis of the characters described below, resulted in one strict consensus tree.

1. **Number of cephalic nerves:** (0) numerous, (1) four nerve cords

   Nemerteans possess cephalic nerves which originate in the brain and run to the anterior (fig. 4a). There are numerous of them in most species studied, but cephalothricid species have been reported to possess only four of them (Gibson 1972). Light microscopy reveals that these actually are nerve cords and part of the central nervous system.

2. **Cephalic nerves arrangement:** (0), nerves form cylinder (fig. 14a), (1) nerves form groups of nerves (fig. 33a)

   In some nemerteans the cephalic nerves form a closed circle which appears as a cylinder.

   This character is not applicable for nerve cords

3. **Cephalic nerves, position of groups of nerves:** (0) around rhynchocoel (fig. 14a), (1) around head lacuna (fig. 33a)

   This character is not applicable for nerve cords

4. **Cephalic nerves, topography:** (0) epidermal, (1) subepidermal

   Depending on the position of the nervous system, the cephalic nerves are located in different body layers.

   This character cannot be applied for single nerves

5. **Cephalic nerves, structure:** (0) contain somata (fig. 8a), (1) somata restricted to brain & medullary cords (fig 33a)

   In Cephalothricidae the cephalic nerves are covered by neuronal cell somata. In hetero-, hoplo- and other palaeonemerteans the neuronal cell somata are inside the brain.

6. **Cephalic nerves, position of cell somata:** (0) form distinct layer (fig. 8a), (1) are distributed along the neurites of the cephalic nerves (fig. 28a)

   This character cannot be applied for cephalic nerves without somata

7. **Position of brain:** (0) in epidermis (fig. 22a), (1) directly below basal lamina of the epidermis (fig. 14a), (2) inside body musculature (fig. 33a)

8. **Partition:** (0) two lobes (fig. 8d), (1) three lobes (figs. 4a, 33c)

   The brain of nemerteans is in its posterior part divided in at least two lobes.

9. **Commissural tracts:** (0) dorsal anterior, (1) ventral anterior, (2) same level

   The two parts of the brain are connected by commissural tracts above or below the rhynchocoel. (fig. 4b)

10. **Commissural tract development:** (0) dorsal neuropil thicker than ventral, (1) ventral neuropil thicker than dorsal, (2) equal

11. **Dorsal commissural tracts:** (0) one (fig. 42b) , (1) more than two (fig. 17b)

    In Tubulanus more than two commissural tracts are present.

12. **Outer neurilemma clearly discernable:** (0) absent (fig. 9a), (1) present (fig. 43c)

The brain of nemerteans may be surrounded by an extracellular matrix (called outer neurilemma). (fig. 4b)

13. **Inner neurilemma:** (0) absent (fig. 43c), (1) present (fig. 8c)

The neuronal cell somata of the brain may be separated from the neuropil by an extracellular matrix (called inner neurilemma).

14. **Patterning of the neurites in the brain neuropil:** (0) absent, (1) present (fig. 50)

In nemerteans the neurites of the brain may be differently arranged

15. **Horizontal arrangement of neurites in the neuropil of the brain:** (0) absent, (1) present (fig. 50)

The neurites in the commissural plexus are horizontally arranged

16. **Vertical arrangement of neurites in the neuropil of the brain:** (0) absent, (1) present (fig. 50)

The neurites connecting the ventral with the dorsal lobe of the brain of hoplonemerteans are vertically arranged

17. **Spherical arrangement of neurites in the dorsal lobe of the brain neuropil:** (0) absent, (1) present (fig. 50)

In hoplonemertea the neurites of the dorsal lobe of the brain form spherical structures

18. **Large cell somata in brain present:** (0) absent, (1) present (figs. 34a, b)

Neuronal cell somata which are at least double the size of the other cell somata

19. **Location of brain relative to mouth opening:** (0) close to mouth opening (fig. 21b) (1) far anterior to mouth opening (fig. 7b)

Far away means that the brain is triple the length from the tip of the head to the brain away from the mouth opening

20. **Ecm branches into the neuropil of the brain:** (0) absent, (1) present (fig. 32b)

In some nemerteans extracellular matrix branches into the brain neuropil.

21. **Neurites of brain neuropil branches into cell somata layer:** (0) absent, (1) present (fig. 32b)

In some nemerteans the neurites of the brain neuropil branches into the cell somata layer

22. **Dorsal lobe of brain in close contact to blood vessel:** (0) absent, (1) present (fig. 51d)

The dorsal lobe of the brain may end in close contact to the blood vessels

23. **Eyes:** (0) absent, (1) present

Some nemerteans possess pigmented eyes as adults (figs. 4a, 48d)

24. **Frontal sense organ:** (0) absent, (1) present (figs. 41c, 54d)

Frontally located sense organ. One dorsal and sometimes two lateral pits. Canal ciliated and short, located close to the tip of the head, sometimes at the level of proboscis pore (fig. 4a)

25. **Composition of frontal sense organ:** (0) one dorsal pit (fig. 54d), (1) one dorsal, two lateral pits (fig. 41c)

In *Oerstedia dorsalis* the frontal organ is composed of one dorsal pit, in heteronemerteans of one dorsal and two lateral pits

This character can only be applied for animals which do not possess a frontal organ

26. **Cerebral organ:** (0) absent, (1) present (figs. 4a, 16a, 33c)

Cerebral organ: sensory structure, composed of a ciliated canal lined with sensory cell which have contact to the brain by fine neurites showing immunoreactivity against α-tubulin. Canal may end in a special layer of neuronal cell somata and sometimes glandular cells. Cerebral organ located anterior, posterior or at same level with brain. Cerebral organ may be in contact with blood vessel. Canals never open dorsally.

27. **Canal innervated by neurites which show immunoreactivity against Tubulin:** (0) absent, (1) present (fig. 26a)

This character can only be applied for animals which do not possess a cerebral organ.

28. **Canal terminates:** (0) anterior to brain (fig. 43a), (1) posterior to brain (fig. 33c),

(2) at the same level (fig. 51c)

This character can only be applied for animals which do not possess a cerebral organ

29. **Opening of canal:** (0) ventral (fig. 49a), (1) lateral (fig. 14d), (2) ventrolateral

(fig. 51b)

This character can only be applied for animals which do not possess a cerebral organ

30. **Canal ends in a special layer of cell somata and gland cells:** (0) absent, (1) present (fig. 33c)

This character can only be applied for animals which do not possess a cerebral organ

31. **Organ close to blood vessel:** (0) absent, (1) present (fig. 33d)

In hetero- and hoplonemerteans the cerebral organ terminates close to the blood vessel, separated by an extracellular matrix.

This character can only be applied for animals which do not possess a cerebral organ

32. **Connection to environment:** (0) slits (33b), (1) pits (51b)

This character can only be applied for animals which do not possess a cerebral organ

33. **Canal below basal lamina of brain:** (0) absent (fig. 33a), (1) present (33a)

In palaeonemerteans possessing a cerebral organ the canal terminates anterior to the basal lamina of the epidermis.

This character can only be applied for animals which do not possess a cerebral organ

34. **Canal course:** (0) elongated (fig. 36d) (1) simple ducts (fig. 26a)

This character can only be applied for animals which do not possess a cerebral organ

35. **Cephalic glands:** (0) absent, (1) present (fig. 28b)

Some nemerteans possess glands with contact to the head lacuna in the tip of the head.

36. **Intraepidermal nerve plexus:** (0) absent, (1) present (fig. 5a)

Nemerteans possess plexus in different part of their body.

37. **Neurite arrangement intraepidermal plexus:** (0) regular (fig. 25b), (1) irregular

The arrangement of the neurites differs between various body tissues. (fig. 5a)

38. **Subepidermal nerve plexus:** (0) absent, (1) present (fig. 5b)

39. **Neurite arrangement subepidermal plexus:** (0) regular (fig. 25a), (1) irregular

40. **Subrastomatogastric nerve plexus:** (0) absent, (1) present (fig. 5a)

41. **Neurite arrangement suprastomatogastric nerve plexus:** (0) regular (fig. 35c), (1) irregular

42. **Intrastomatogastric nerve plexus:** (0) absent, (1) present (fig. 5a)

43. **Neurite arrangement intrastomatogastric nerve plexus:** (0) regular (fig. 35c), (1) irregular

44. **Commissural plexus:** (0) absent, (1) present (fig. 5a)

45. **Neurite arrangement commissural nerve plexus:** (0) regular (fig. 20d), (1) irregular

46. **Dorsal nerve:** (0) absent, (1) present (figs. 5b, 8e)

In some nemerteans a dorsal nerve originates in the dorsal commissural tract

47. **Origin of dorsal nerve:** (0) epidermal (fig. 22d), (1) subepidermal (fig. 14d), (2) inside musculature (fig. 33c)

This character is not applicable for animals without dorsal nerve.

48. **Further course of dorsal nerve:** (0) epidermal (fig. 22d), (1) subepidermal (figs. 31b-d), (2) inside musculature

note: in *Carinoma* and Cephalothricidae the dorsal nerve originates in the dorsal commissural tract and is embedded in the musculature. To the posterior the nerve runs below the basal lamina of the epidermis

49. **Secondary dorsal nerve:** (0) absent, (1) present (fig. 5b, *Carinina ochracea*)

In *Carinina ochracea* a secondary dorsal nerve runs above the rhynchocoel

50. **Ventral nerve:** (0) absent, (1) present (figs. 5b, 8e))

In some nemerteans a ventral nerve originates in the ventral commissural tract.

51. Proboscis nerves number: (0) two (fig. 8d), (1) more than two (fig. 51b)

Proboscis nerves run along the proboscis

52. Proboscis nerve origin: (0) ventral commissural tract (fig. , (1) dorsal commissural tract (fig. 53b)

In hetero- and palaeonemerteans the proboscis nerves originate in the ventral commissural tract, while they originate in the dorsal commissural tract in some hoplonemerteans

53. Esophageal nerves origin: (0) ventral commissural tract (fig. 30b), (1) ventral lobe of brain

In heteronemerteans the esophageal nerves originate in the posterior part of the ventral lobe of the brain.

54. Medullary cord: topography: (0) epidermal (fig. 22d), (1) subepidermal (fig. 28f), (2) in musculature (fig. 56f)

Medullary cords originate in the ventral lobe of the brain and run to the posterior of the animal (fig. 4a).

55. Medullary cord: position of neuronal cell somata: (0) u-shaped, cap to the interior (fig. 57c), (1) u-shaped cap to the exterior (fig. 33d), (2) dorsally and ventrally (fig. 31d).

56. Medullary cord: inner neurilemma: (0) absent (fig. 54c), (1) present (fig. 31d).

57. Medullary cord: outer neurilemma: (0) absent (fig. 8f), (1) present (fig. 54c)

58. Medullary cord in close association with nephridia: (0) absent, (1) present (fig. 55b)

In hoplonemerteans the medullary cords are in close association with the nephridia

59. Accessory lateral nerve: (0) absent, (1) present (fig. 54c)

A nerve which originates in the brain and runs above the lateral medullary cord until they join.

60. Circular nerves connecting the medullary cord: (0) absent, (1) present (fig. 10e)

In *Procephalothrix filiformis*, circular nerves connect the medullary cords

61. Circular nerves serial arranged: (0) absent, (1) present (fig. 10e)

In *Procephalothrix filiformis* the circular nerves which connect the medullary cords are serial arranged

4.3 Phylogenetic analysis

Nemerteans possess typical spiralian brains in being composed of a central neuropil and a layer of neuronal cell somata which are homogeneously distributed around the neuropil (Richter et al. 2010), but their position inside the lophotrochozoan tree is still under debate.

4.3.1 Choice of outgroup

Several spiralian taxa are supposed to be the next relatives to nemerteans. The conditions concerning the nervous system differ considerably between taxa. Since similar conditions are also found in various basal branching nemerteans, two or three nemertean taxa are possible the ancestor of all nemerteans. There is no doubt in literature (e. g. Bürger 1895, Coe 1943, Gibson 1972, Thollesson & Norenburg 2003) that members of the palaeonemerteans are the most basal branching taxa of nemerteans.

Recent studies place nemerteans in the vicinity of brachiopods, annelids, molluscs (e.g. Giribet et al. 2000, Dunn et al. 2008, Heijnol et al. 2009, Paps et al 2009) and in the classical view they are closely related to platyhelminthes (e.g. Bürger 1895, Hyman 1951, Nielsen 2001).

Iwata (1969) and Bürger (1895) based their discussion on the last common ancestor of all nemerteans, among other characters, on the position of the nervous system inside the body wall. In *Carinina ochracea* the central nervous system is located inside the epidermis; in Cephalothricidae inside the musculature. Regarding the location of the nervous system of possible non nemertean outgroups both situations present in above mentioned nemerteans are found. In annelids (Golding 1992) and molluscs (Hyman 1967) the nervous system is located in the musculature and in brachiopods it is located inside the epidermis (James 1998). Moreover also the condition found in *Tubulanus* (directly below basal lamina of epidermis) is present in annelids (Golding 1992).

The unknown outgroup conditions influence the topology of the tree insofar as two or three ingroup taxa (*Carinina ochracea,* Cephalothricidae*, Tubulanus*) are possible the most basal branching taxa.

4.3.2 Nemertean interrelationship

The phylogenetic analyses based on morphological elements of the nervous system always provide a monophyly of hetero- and hoplonemerteans. Together these two taxa form the monophyletic clade *Neonemertea* (Thollesson & Norenburg 2003). This was also found in the study by Andrade et al (2011). Although this relationship is not very robust in any of these studies, the two recently published studies are congruent with the results presented here.

Palaeonemerteans sensu stricto (comprising *Tubulanus*, Cephalothricidae, *Callinera monensis*, *Carinoma mutabilis and Carinina ochracea* in this study) are paraphyletic in the present study as well as in the study by Thollesson and Norenburg (2003). The nervous system of palaeonemerteans shows the highest variation among investigated taxa. The position of the central nervous system inside the body wall reaches from intraepidermal (*Carinina*) to subepidermal (*Tubulanus*) to intramuscular (Cephalothricidae, *Carinoma*). A common morphological element of the nervous system of palaeonemerteans (*Carinina, Carinoma, Tubulanus* + *Callinera monensis*) excluding Cephalothricidae is the circular arrangement of the cephalic nerves around the head lacuna. This condition is neither found in hetero- nor in hoplonemerteans. Cephalothricidae possess four solitary cephalic nerve cords. The cephalic nerves of hetero- and hoplonemerteans are arranged in groups around the rhynchocoel. In investigated Cephalothricidae the cephalic nerve cords are covered by a layer of neuronal cell somata, due to that condition the cephalic nerves are now termed cephalic nerve cords.

The data of the nervous system as well as other morphological elements like nephridia (Bartolomaeus & von Döhren 2010), sperm ultrastructure (von Döhren et al. 2010) and molecular data (e.g. Sundberg et al. 2001, Thollesson & Norenburg 2003) provide no indications which allow the characterization of palaeonemerteans as monophyletic. Since characters of different palaeonemertean species are also present in the possible sistergroups of nemerteans, at least some of the characters of palaeonemerteans are plesiomorphic. Due to these arguments I suppose that a taxon Palaeonemertea should be to rejected.

A classification similar to the present results was already purposed by Iwata 1960. He established a new taxon called Archinemertea for the Cephalothricidae and separated them from the remainder of the palaeonemerteans. He based his classification on the location of the nervous system inside the musculature, on the position of the brain relative to the mouth, on the absence of a cerebral organ, the arrangement of the cephalic nerves and other non nervous morphological characters. But Iwata´s classification was controversially discussed and is neglected by most authors (e.g. Gibson 1972, Thollesson & Norenburg 2003, Andrade et al. 2011).

In the present analysis *Tubulanid* nemerteans (sensu stricto) are paraphyletic. This is also found by Andrade et al. 2011. Here *Carinina ochracea* is closer related to *Carinoma* than to *Tubulanus*. *C. ochracea* is unusual with respect to the location of the nervous system. It is the only nemertean taxon known where the nervous system is inside the epidermis. The morphology of the cerebral organ differs also to the situation found in *Tubulanus* as the canal is elongated and proceeds dorsally. Additionally the canal passes through the neuronal cell somata of the brain.

Tubulanus and *Callinera monensis* are always closely related, although *Tubulanus* possess a cerebral organ and *Callinera monensis* does not. An autapomorphy for this taxon is the location of the nervous system just beneath the basal lamina of the epidermis (subepidermal).

The ingroup relationship among hetero- and hoplonemerteans is not resolved. This may be due to the fact that the nervous system appears very homogeneously in these two taxa.

If the tree is artificially rooted with heteronemerteans, palaeonemerteans become monophyletic and closer related to hoplonemerteans or vice versa if the tree is rooted with hoplonemerteans. This show the difficulties in choosing the correct outgroup. The reliability of a tree heavily depends on choosing the appropriate taxon for rooting the tree. Not only if we want to infer phylogeny of a single taxon but also if we want to infer phylogeny of higher metazoan taxa we have to compare the morphological elements present in the last common ancestor of the particular taxa we investigate. That is why it is very important to be able to infer the morphological elements present in the ground pattern of taxa chosen for phylogenetic analysis.

4.4 Evolution of the nervous system

According to the trees obtained in the phylogenetic analysis the evolution of the elements of the nervous system of nemerteans is discussed. Since a final conclusion concerning the most basal branching nemertean taxon of is still wanting, some hypotheses are presented below.

4.4.1 Brain, nerve cords and peripheral nervous system

According to the tree rooted with Cephalothricidae (fig. 59) I suggest a following evolutionary scenario concerning the elements of the nervous system:

Basically the nemertean nervous system comprised a four lobed brain and two lateral medullary cords. The nervous system is located inside the musculature and a dorsal and a ventral nerve are present. Several nerve plexus are discernable in various parts of the body. The cerebral organ arose in the stem lineage of *Carinina ochracea*, *Carinoma mutabilis*, *Tubulanus* + *Callinera monensis* and Neonemertea, and is an autapomorphy for this evolutionary lineage. In this analysis *C. ochracea* is the first branching species of nemerteans possessing a cerebral organ and the intraepidermal position of the nervous system must be regarded as an autapomorphy for the taxon.

Rooting the tree with *Carinina ochracea* (fig. 59) implied the following assumptions for the primary structure of the nemertean nervous system: the conditions found in *Carinina ochracea* represent the ground pattern and the position of Neonemertea and *Tubulanus* + *Callinera monensis* does not change. Only *C. ochracea* and Cephalothricidae change positions.

Nemertean nervous system basically comprised a four lobed brain and two lateral medullary cords. The nervous system is located basiepidermal and a dorsal nerve is present. The cerebral organ as an epidermal canal lined with sensory cells which are connected to the brain, is developed. Several nerve plexus are discernable in various parts of the body.

This hypothesis was purposed by Bürger (1895) and justified by the basiepidermal position of the nervous system. He supposed that the nervous system migrated inwards in "higher" nemerteans. Rooting the tree with *C. ochracea* would affect the morphological elements present in the ground pattern of nemerteans but not the evolutionary scenario described below.

The presence of a distinct and clearly discernable outer neurilemma surrounding the brain and medullary cords is an autapomorphy for a taxon comprising *Carinoma*, *Tubulanus* and Neonemertea. In the stem lineage of Neonemertea the canal that connects the cerebral organ with the environment terminates below the basal lamina of the epidermis, which is not the case in *Carinina ochracea* and *Tubulanus;* here it terminates anterior to the basal lamina of the epidermis. Monophyly of lineid heteronemerteans is supported by the position and morphology of the cerebral organ and the possession of cephalic slits. Additionally in investigated heteronemerteans the brain is tripartite and very prominent neuronal cell somata are present. The composition of the frontal organ (two lateral and one dorsal pit) is also unique to investigated heteronemerteans.

Only in investigated hoplonemerteans the lateral medullary cords are in close association with the nephridial system as well as the dorsal lobe of the brain is in close proximity to the blood vessels. The numbers of proboscidial nerves raises up to 16 in *Amphiporus* (Hoplonemertea) while in investigated palaeo- and heteronemerteans only two proboscidial nerves are present. Only in hoplonemertea the neurites of the dorsal lobe of the brain form spherical structures. Dorsal and ventral nerves are absent in hoplonemertea, while present in palaeo- and heteronemerteans.

4.4.2 Cerebral organs

I suppose that one key question to resolve nemertean phylogeny is whether the cerebral organ is homologous or not and whether it has been reduced in certain lineages or not. I determine the cerebral organ as a ciliated epidermal canal or duct lined with sensory cells which are connected to the brain by fine neurites showing immunoreactivity against α-tubulin. The canals may taper to ducts which end in a layer of neuronal cell somata and glandular cells internal to the basal lamina of the epidermis.

The canal terminates in various positions relative to the brain in different nemertean taxa. In *Tubulanus* the duct terminates posterior to the division of the brain into a dorsal and ventral lobe. In most hoplonemerteans it terminates anterior to the brain, although in *Prosorhochmus claperedii* the canal terminates at the same level with the brain. In heteronemerteans the canal ends posterior to the brain. Because of these different arrangements many authors (e.g. Bürger 1895, Gibson 1972) concluded, that the cerebral organ could have evolved independently in nemertean taxa. But the different location of the canal relative to the brain in hoplonemerteans may also be due to the fact, that during ontogenesis the mouth opening is being positioned more anterior and shares a common opening with the proboscis (Gibson 1972).

I think that at least the epidermal canals, which are lined with sensory cells that are connected to the brain, of the cerebral organ can be homologized in all investigated nemerteans possessing a cerebral organ (*C. ochracea*, *Tubulanus* + *Callinera monensis* and Neonemertea). If the cerebral organs are not homologous and present in the last common ancestor or in a stem lineage comprising nemerteans with a cerebral organ (*Tubulanus*, *Carinina ochracea,* Neonemertea) an independent evolution would have appeared three times in *Tubulanus*, *Carinina ochracea* and Neonemertea. This scenario is in the view of the present results not the most parsimonious and should be rejected.

More likely is that the cerebral organ arose once in a stem lineage comprising nemerteans with a cerebral organ or is present in the ground pattern of nemerteans. If the cerebral organ was present in the ground pattern it would have been reduced three times independently in Cephalothricidae, *Callinera monensis* and *Carinoma mutabilis*. If the cerebral organ was not present in the ground pattern and arose in a stem lineage comprising nemerteans with a cerebral organ (*Carinina ochracea*, *Tubulanus* + *Callinera monensis* and Neonemertea) it would have been only reduced twice independently in *Carinoma mutabilis* and *Callinera monensis*.

According to the resulting trees of this analysis two evolutionary hypotheses concerning the cerebral organ are possible:

1 The cerebral organs (comprising epidermal canals lined with sensory cells, which are connected to the brain by neurites showing immunoreactivity against α-tubulin) were present in the stem lineage of all nemerteans. Since any comparable structure is supposedly missing in other spiralians, it is an autapomorphy for this taxon. It is reduced three times independently in Cephalothricidae, *Callinera monensis* and *Carinoma mutabilis*.

2 The cerebral organs are not present in the last common ancestor of all nemerteans and evolved once in the stem lineage comprising palaeonemerteans with a cerebral organ (*Carinina*, *Tubulanus*) and the Neonemertea sensu Thollesson & Norenburg (2003) and is reduced twice independently in *Callinera monensis* and *Carinoma mutabilis.*

Generally the cerebral organ consists of simple epidermal tube like ducts (*Tubulanus*) or slightly elongated epidermal canals (*Carinina ochracea*) which are densely ciliated and terminate anterior to the basal lamina of the epidermis. In the stem lineage of neonemerteans the canal is elongated and terminates in a layer of special neuronal cell somata and glandular cells underneath the basal lamina of the epidermis. This goes along with a higher complexity of the brains in terms of neurite arrangement in hoplonemerteans and a tripartition of the brain in lineid heteronemerteans. The elongation of the canal which is most developed in lineid heteronemerteans and achieved by convolution, raises the number of receptive cells lining the canal and thus the receptive surface is expanded. The epidermal canals taper to a ducts which end in a layer of neuronal cell somata and glandular cells close to the blood vessels in heteronemerteans and some hoplonemerteans, merely being separated by a layer of extracellular matrix. Due to this arrangement the cerebral organ is supposed to have a neurosecretory or osmoregulatory function (e.g. Scharrer 1941, Ling 1969, 1970, Ferraris 1978). The study by Amerongen & Chia (1982) demonstrates that the cerebral organ plays a major role in hunting.

I suggest that nemerteans possessing such a well developed sensory organ are potentially able to catch more active prey that does not have to be necessarily in their closer vicinity. As shown by Thiel (1998) and Amerongen & Chia (1982) hetero- and hoplonemerteans are able to follow the tracks of their bait. All investigated members of the former palaeonemertean group possessing a cerebral organ are mainly endobenthic organism in contrast to the investigated hetero- and hoplonemerteans which are mainly epibenthic animals. *Tubulanus polymorphus* and *Tubulanus superbus* have been found in the eelgras meadows of Roscoff.

Since these animals are comparable huge (up to 20 cm long and 3 cm thick, own observation) and their tubes are interwoven with the roots of the eelgras, it is not likely that the animals actively hunt their prey as their epibenthic relatives do. Furthermore all these animals lack eyes as adult organism what is may also be an indicator for a permanent endobenthic live cycle as adults. Since Neonemertea are the by far the most species rich group of nemerteans the elongation of the canal and the connection of the cerebral organ to the blood vessels in the stem lineage of this taxon may be a key element in the radiation of this group.

According to the resulting trees an evolution of the cerebral organ in a stem lineage comprising nemerteans with a cerebral organ is the most parsimonious assumption. Due to that, at least remainders or Anlagen of the cerebral organ should be found in those nemerteans which lack a cerebral organ as adults (*C. mutabilis*, *C. monensis*). Bürger reports, that in *Carinoma armandi* the cerebral organ is only rudimentary, comprising a non glandular part and a ganglionic structure which is connected to the dorsal lobe of the brain. He considered this as the nervous portion of the cerebral organ (Bürger 1895).

4.5 Comparison with outgroup

Ciliated sensory structures which are supposed to have a chemosensitive function are found in platyhelminthes, Mollusca and in Annelida. Platyhelminthes possess ciliated sense organs on the tip of the head, which are supposed to have a chemoreceptive function (Rieger et al. 1991). In Annelids nuchal organs are present. These are small chambers which are situated at the prostomium of the animals. The chamber is lined with sensory cells which are connected to the brain by neurites (Purscke 1996). Aplacophoran molluscs possess a sensory organ on the tip of their head which is also supposed to have a chemoreceptive function (Haszprunar 1987). The function, morphology and position of these sensory structures are comparable to those of nemerteans but whether these are homologous to the cerebral or frontal organs remains unknown.

In the ground pattern of nemerteans several nerve plexus are present of which some have also been described for other spiralians. For example subepidermal nerve plexus have been found in platyhelminthes (Baguna & Ballester 1978, Reuter & Gustafsson 1995), polychaetes (Orhage & Müller 2005, Golding 1992) and phoronids (Herrmann 1997) and intraepidermal nerve plexus have been described for Platyhelminthes (Reuter & Gustafsson 1995).

Nothing is known about the arrangement of the neurites in the different nerve plexus and thus a homologization of these nervous elements is at present state of knowledge not possible. Medullary cords have been described in Platyhelminthes (Reuter & Gustafsson 1995), basal molluscs (Moroz et al 1994, Eernisse & Reynolds 1994, Scheltema et al. 1994, Todt et al. 2008, Faller & Loesl 2008) and polychaetes (Golding 1992, Orrhage & Müller 2005).

In hoplonemerteans the neurites of the brain neuropil form different patterns. In the commissural plexus they are horizontally arranged. The neurites which connect the dorsal with the ventral lobe of the brain are vertically arranged. In the dorsal lobe of the brain spherical structures are visible. This patterning of different neurites in the brain is only visible in Azan staining and not with immunostaining techniques. A patterning of different neurites in the brain (subcompartimentation) is present in vagile polychaetes (Heuer & Loesl 2004, Loesl & Heuer 2010) and arthropods (Strausfeld 1995) where a clear subdivision of different brain parts is visible. But this kind of subcompartimentation is not homologous to the conditions found in hoplonemertean brains.

A serial arrangement of circular nerves connecting the medullary cords is found in *Procephalothrix filiformis*. In *Carinina ochracea* the neuropil of the medullary cords are connected to the neurites of the subepidermal nerve plexus with regular arranged branches of neurites showing immunoreactivity against α-tubulin. In addition, at the spots where the neurites of the subepidermal nerve plexus branch of, a spherical concentration of neurites occur. Since a serial arrangement of particular nerves is also present in platyhelminths (Reuter & Gustafsson 1995), annelids (Golding 1992) and basal molluscs (Hyman 1967) I suggest this as an element of the last common ancestor of nemerteans and spiralians.

As described above, Iwata (1969) and Bürger (1895) based their discussion on the last common ancestor of all nemerteans, among other characters, on the position of the nervous system relative to the integument layer. In platyhelminths (Reuter & Gustafsson 1995), sipunculids (Rice 1993) as well as in kamptozoans (Nielsen & Jespersen 1997) the nervous system is located subepidermally. In basal molluscs (Hyman 1967) the nervous system is located in the longitudinal musculature and in brachiopods it is located basiepidermally (James 1997). In annelids the nervous system may be located subepidermally (*Owenia fusiformis*, Coulon and Bessone 1997), or in the musculature (*Nereis*, Golding 1992). But there is an ongoing debate about the phylogeny of annelids (Rousse & Fauchald 1997, Struck et al. 2010) and thus it is not known which condition represents the ancestral state.

The position of the nervous system relative to the body wall layer in above mentioned taxa is also found in the possible basalmost branching nemerteans. In *Carinina ochracea* the central nervous system is located inside the epidermis; in Cephalothricidae inside the longitudinal musculature and in *Tubulanus* subepidermal. Due to the unknown sistergroup, a conclusion about the morphological elements present in the ground pattern is not possible. A final conclusion concerning the basalmost branching nemertean taxa heavily relies on the topology of the spiralian tree and the conditions found in the sistergroup of nemerteans.

This example shows that it is crucial to know the morphological elements present in the ground pattern of investigated taxa, if we want to infer phylogeny. Although a final conclusion about the morphological elements present in the ground pattern of nemerteans is still not possible, the results show that the nervous system is a valuable source of data to infer nemertean phylogeny. If the morphological elements present in the ground pattern of the nemertean sistergroup are known, it will be possible to infer nemertean phylogeny at the base of the present data matrix.

4.6 Conclusions

The fine structure of the nervous system of nemerteans differs considerably between evolutionary lineages. Three mayor clades are discernable: Palaeo- hetero- and hoplonemerteans. Palaeonemerteans are paraphyletic, while hetero- and hoplonemerteans are monophyletic with good support. A taxon comprising hetero- and hoplonemerteans (Neonemertea) is weakly supported. Since the conditions concerning the location or structure of the nervous system in the most basal branching taxa are also found in several closely related spiralian taxa, like platyhelminthes, molluscs, annelids and brachiopods a final conclusion about the most basal branching taxon of nemerteans is still not possible. Potential taxa for rooting the tree are the Cephalothricidae, *Tubulanus* and *Carinina ochracea*. Rooting the tree with these different taxa influences the ground pattern of nemerteans but not the overall topology of the tree. Due to the unknown sistergroup of nemerteans, a final conclusion concerning the morphological elements of the nervous system present in the ground pattern of nemerteans is not possible. Compared to other recently published results the nervous system, although being just one organ system turned out to be a reliable morphological system to infer nemertean phylogeny.

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6 Summary

Most described nemerteans are marine benthic hunters. Thus they possess a well developed nervous system and several sensory structures. Basically, the nervous system comprises a four lobed brain, two main lateral nerves, some minor nerves and various nerve plexus. The brain and lateral nerve cords are composed of a central neuropil and a layer of neuronal cell somata, which are homogeneously distributed around the neuropil. Nemerteans may possess ciliated chemical sensory organs which are located on the tip of the head. One of these organs is the cerebral organ which is supposed to be homologous in nemerteans and thus may be a valuable morphological element to infer nemertean ingroup relationship. Formerly nemerteans fell into three major groups, the palaeo- hetero- and hoplonemertea. But this classification is in recent years controversially discussed. Especially molecular studies regard palaeonemerteans to be paraphyletic and hetero- and hoplonemerteans to be an evolutionary lineage called neonemertea.

Recently a lot of studies are available dealing with the nervous system of members of the spiralians. Since the nervous system of nemerteans was often neglected in phylogenetic analysis in latest publications, the nervous system of nemerteans was reinvestigated using classical and new techniques. Azan staining was chosen to reveal the overall anatomy of the nervous system and additionally immunohistochemical methods were applied to reveal the fine structure. Aim of this study is to reconstruct the conditions present in the ground pattern of nemertean nervous system. Not only to infer nemertean phylogeny but also to be able to infer the position of nemerteans in the spiralian tree. Therefore several specimen belonging to the supposed basal group of nemerteans were investigated. Additionally specimens which are supposed to show derived characters were investigated to get an overall impression of the different conditions concerning the nervous system in nemertean groups. A careful analysis of the nervous system showed considerable differences in the fine structure of the nervous system of even closely related species. For example the arrangement of neurites in the brain differs between higher nemertean groups. Additionally some nemerteans do possess a conspicuous cerebral organ and others not. The phylogenetic analysis of the morphological elements derived from the nervous system show that palaeonemerteans are paraphyletic.

Hetero- and hoplonemerteans are monophyletic and can be regarded as an evolutionary lineage, although with weak support. A final conclusion concerning the morphological elements present in the ground pattern of nemerteans is still not possible, due to the unknown outgroup conditions.

6 Zusammenfassung

Die meisten der beschriebenen Nemertinenarten sind benthische Jäger. Deshalb sind sie mit einem gut entwickeltem Nervensystem und verschiedenen Sinnesorganen ausgestattet die es Ihnen erlaubt mit Ihrer Umwelt zu interagieren.

Das Nervensystem der Nemertinen besteht grundsätzlich aus einem 4-lobigen Gehirn, zwei lateralen Hauptnerven und einigen Nebennerven sowie mehreren Nervenplexus. Das Gehirn und die beiden lateralen Markstränge bestehen aus einem zentralen Neuropil und die es umgebenden neuronalen Zellsomata. Die Somata sind gleichmässig über das Neuropil verteilt. Nemertinen besitzen am Vorderende ciliäre chemische Sinnesorgane. Eines dieser Organe, das Cerebralorgan, ist möglicherweise ein Merkmal um die Innengruppenphylogeny der Nemertinen nachvollziehen zu können.

Früher wurden Nemertinen in drei Hauptgruppen aufgeteilt, die Palaeo- Hetero- und Hoplonemertinen. Heutzutage wird diese Klassifikation angezweifelt. Besonders molekulare Daten zeigen, dass Palaeonemertinen paraphyletisch sind und Hetero- und Hoplonemertinen eine Abstammungsgemeinschaft bilden, die Neonemertea genannt wurde.

In den letzen Jahren sind viele Arbeiten zum Nervensystem verschiedener Spiralier erschienen. Das Nervensystem der Nemertinen wurde in den phylogenetischen Untersuchungen der letzten Jahre oft nicht berücksichtig. Deshalb wurde das Nervensystem der Nemertinen mit klassischen und modernen Methoden neu untersucht. Azan Färbung wurde gewählt um einen Überblick über das gesammte Nervensystem zu bekommen. Immunohistologie wurde zusätzlich gewählt, um die Feinstruktur des Nervensystem nachvollziehen zu können.

Ziel dieser Arbeit ist die morphologischen Elemente zu charakterisieren, welche im Grundmuster der Nemertinen vorhanden sind. Nicht nur um die Innengruppenphylogenie der Nemertinen aufzuklären, sondern auch um die Position der Nemertinen im Stammbaum der Spiralier nachvollziehen zu können. Deshalb wurden Nemertinen untersucht die als ursprünglich betrachtet werden. Um einen Gesamteindruck der Zustände im Nervensystem der Nemertinen zu bekommen, wurden zusätzlich Arten untersucht die abgeleitete Merkmale aufweisen. Eine gründliche Untersuchung des Nervensystem zeigt beträchtliche Unterschiede in der Feinstruktur zwischen sogar nah verwandten Arten. So sind zum Beispiel die Neurite im Neuropil des Gehirns der Hoplonemertinen verschiedenartig angeordnet. Zusätzlich besitzen manche Nemertinen auffällige Sinnesorgane und andere nicht.

Die phylogenetische Analyse der Merkmale des Nervensystem ergaben, daß Palaeonemertinen paraphyletisch sind, während Hetero- und Hoplonemertinen jeweils gut unterstützt monophyletisch sind und zusammen eine schwach unterstütze Abstammungsgemeinschaft bilden. Eine abschliessende Aussage über die Zustände im Grundmuster ist noch nicht möglich, da die Schwestergruppe der Nemertinen nicht eindeutig festzulegen ist. Ist diese aber bekannt, wird es möglich sein, anhand der hier vorgestellten Merkmalsmatrix, die Phylogenie der Nemertinen nachvollziehen zu können

Species/ Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Procephalothrix filiformis	1	1	-	-	0	0	2	0	0	1	0	0	1	1	1	0	0	1	1	1	1	0	0	0	-	0	-	-	-	-
Cephalothrix linearis	1	1	-	-	0	0	2	0	0	1	0	0	1	1	1	0	0	1	1	1	1	0	0	0	-	0	-	-	-	-
Tubulanus polymorphus	0	0	1	1	1	-	1	0	0	1	1	1	0	1	1	0	0	0	0	1	1	0	0	0	-	1	?	2	1	0
Tubulanus superbus	0	0	1	1	1	-	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	-	1	?	2	1	0
Carinina ochracea	0	0	1	0	1	-	0	0	1	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	-	1	1	1	1	0
Carinoma mutabilis	0	0	1	1	1	-	2	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	0	0	-	0	-	-	-	-
Callinera monensis	0	0	1	1	0	1	1	0	2	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	-	0	-	-	-	-
Lineus ruber	0	1	0	-	1	-	2	1	2	2	0	1	1	1	1	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1
Micrura purpurea	0	1	0	-	1	-	2	1	0	1	0	1	1	1	1	0	0	1	0	1	0	0	1	0	-	1	?	1	1	1
Riseriellus occultus	0	1	0	-	1	-	2	1	0	1	0	1	1	1	1	0	0	1	0	1	0	0	1	1	1	1	?	1	1	1
Amphiporus lactifloreus	0	1	0	-	1	-	2	0	1	1	0	1	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	2	1
Amphiporus imparispinosus	0	1	0	-	1	-	2	0	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	0	0	1	?	0	2	1
Prosorhochmus claperedii	0	1	0	-	1	-	2	0	1	1	0	1	0	1	1	1	1	0	0	0	0	1	1	1	-	1	1	2	1	1
Oerstedia dorsalis	0	1	0	-	1	-	2	0	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	1	0	1	?	0	0	1
Nemertopsis bivittata	0	1	0	-	1	-	2	0	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	0	-	1	?	0	0	1
Emplectonema gracile	0	1	0	-	1	-	2	0	1	1	0	1	0	1	1	1	1	0	0	0	0	1	1	0	-	1	?	0	0	1

Species/ Character	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
Procephalothrix filiformis	-	-	-	-	0	1	0	?	?	?	?	?	?	?	?	1	2	1	0	1	0	0	0	2	2	1	0	0	0	1	1
Cephalothrix linearis	-	-	-	-	0	?	?	?	?	?	?	?	?	?	?	1	2	1	0	1	0	0	0	2	2	1	0	0	0	?	?
Tubulanus polymorphus	0	1	0	1	0	1	0	1	0	1	-	1	1	1	0	1	1	1	0	0	0	-	0	1	2	0	1	0	0	?	?
Tubulanus superbus	0	1	0	1	0	?	?	?	?	?	?	?	?	1	?	1	1	1	1	0	0	0	0	1	2	1	1	0	0	?	?
Carinina ochracea	0	1	0	0	0	1	0	1	0	1	-	1	1	?	?	1	0	0	0	0	0	-	0	0	2	1	0	0	0	?	?
Carinoma mutabilis	-	-	-	-	0	?	?	?	?	?	?	?	?	?	?	1	2	1	0	0	0	0	0	2	2	0	1	0	0	?	?
Callinera monensis	-	-	-	-	1	?	?	?	?	?	?	?	?	?	?	1	1	1	0	0	0	-	0	1	2	1	1	0	0	?	?
Lineus ruber	1	0	1	0	0	1	0	1	0	?	?	1	1	1	0	1	2	2	0	0	0	0	1	2	1	1	1	0	0	?	?
Micrura purpurea	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	1	2	2	0	0	0	0	0	2	2	1	1	0	0	?	?
Riseriellus occultus	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	1	2	2	0	0	0	0	0	2	1	1	1	0	0	?	?
Amphiporus lactifloreus	1	1	1	0	0	1	0	1	?	1	?	1	1	?	-	0	-	-	0	0	1	1	?	2	0	0	1	1	0	?	?
Amphiporus imparispinosus	1	1	1	0	0	?	?	?	?	?	?	?	?	?	?	0	-	-	0	0	1	0	?	2	2	0	1	1	0	?	?
Prosorhochmus claperedii	1	1	1	0	1	?	?	?	?	?	?	?	?	?	?	0	-	-	0	0	1	?	?	2	2	0	1	1	0	?	?
Oerstedia dorsalis	1	1	1	0	1	?	?	?	?	?	?	?	?	?	?	0	-	-	0	0	1	1	?	2	2	0	1	1	1	?	?
Nemertopsis bivittata	1	1	1	0	1	?	?	?	?	?	?	?	?	?	?	0	-	-	0	0	1	1	?	2	1	0	1	1	0	?	?
Emplectonema gracile	1	1	1	0	1	?	?	?	?	?	?	?	?	?	?	0	-	-	0	0	1	1	?	2	2	0	1	1	0	?	?